The Toxicity of Hexythiazox to Twospotted Spider Mite (*Tetranychus urticae* Koch) Adults and Eggs

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A thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Applied Science
in the
University of Canterbury

by

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Lincoln College

1988

Abstract of a thesis submitted in partial fulfilment
of the requirements for the Degree of M.Appl.Sc.

THE TOXICITY OF HEXYTHIAZOX TO TWOSPOTTED SPIDER MITE (*TETRANYCHUS URTICAE* KOCH) ADULTS AND EGGS.

by J.W.M. Marris

The toxicity of hexythiazox (tradename Nissorun) to adult female twospotted spider mites (*Tetranychus urticae* Koch) and their eggs was investigated in laboratory studies.

Hexythiazox was found to be relatively non-toxic to adult female *T. urticae*. The calculated LC_{50} value was 59 times that of the suggested field application rate (2.5×10^{-3}% a.i.). Exposure of adult female *T. urticae* to direct sprays and residues on leaf surfaces both caused chemosterilisation of the mites. Eggs produced following treatment failed to hatch. However, this effect was temporary and following removal of the adult female mites from hexythiazox residues, percentage hatch rose to levels equivalent to that of the controls.

A leaf disc method was used to examine the ovicidal activity of hexythiazox. Baseline toxicity data for hexythiazox on *T. urticae* eggs were determined. LC_{50}'s of 1.6×10^{-4}% a.i. for direct spray and 3.2×10^{-4}% a.i. for residue exposure were calculated.

The effect of post-treatment temperature on toxicity of hexythiazox to eggs was investigated. An inverse relationship was found to occur over the temperature range of 15 to 30°C. Eggs maintained at 15°C were 8.7 times more susceptible than eggs maintained at 30°C.

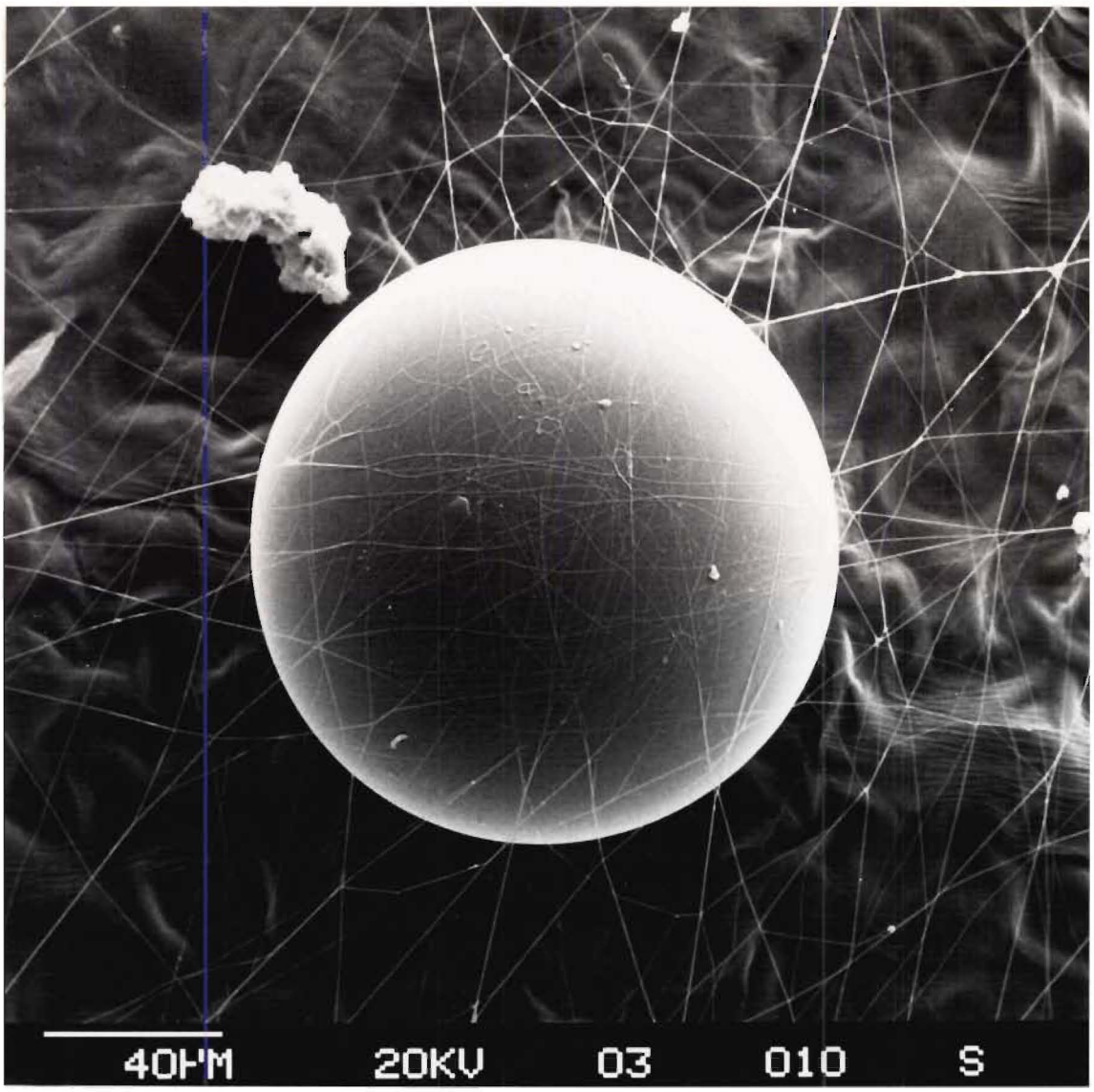
The effect of egg age, at the time of treatment, on toxicity of hexythiazox to eggs was investigated. An inverse relationship was observed. Eggs treated at 48-72 hours were four times more tolerant than eggs treated at 0-6 hours. However, a greater than 100 fold increase in tolerance in eggs treated at 72-96 hours over the previous age group (48-72 hours) occurred.

The effect of leaf type on the toxicity of hexythiazox to *T. urticae* eggs was examined. Five leaf types were used: broad bean (*Vicia faba*, cv. 'Exhibition Long Pod'), strawberry (*Fragaria X ananassa*, cv. 'Red Gauntlet'), raspberry (*Rubus idaeus*, cv. 'Glen Prosen') and two apple cultivars (*Malus* sp. cv. 'Red Delicious' and 'Granny Smith'). Significant differences in toxicity between the leaf types occurred. Hexythiazox was most toxic to eggs laid on broad bean leaves, least toxic to eggs laid on the apple cultivars, and of intermediate toxicity to eggs laid on strawberry and raspberry.

Microscopic examination of eggs treated with a lethal dose of hexythiazox, showed that embryos reach an advanced stage of development before dying. Killed embryos were indistinguishable from newly emerged larvae.

Keywords: Hexythiazox, twospotted spider mite (*Tetranychus urticae*), ovicide, toxicity tests

FRONTISPIECE: Scanning electron micrograph of a twospotted spider mite egg laid on a broad bean leaf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CHAPTER 1
GENERAL INTRODUCTION

The twospotted spider mite (TSM), *Tetranychus urticae* Koch, is a worldwide pest of numerous horticultural crops. In New Zealand TSM is a particular pest of treefruit, berryfruit, glasshouse and ornamental crops. Damage is caused by mites feeding on plant foliage which causes leaf bronzing. Feeding damage may result in water stress which can cause reduced growth and yield loss (van de Vrie *et al.* 1972).

Control of TSM is based largely on the use of acaricidal sprays. Effective control of TSM may be achieved by timing spray applications to coincide with the most susceptible stages. However, as generations overlap sprays may become less effective (Chapman, 1986). Thus a regular schedule of miticide applications may be necessary.

A major problem with chemical control of TSM and other tetranychid mites is the continued development of resistance to a wide range of chemical groups (Cranham and Helle, 1985). This has necessitated the development of further chemical groups with novel modes of action.

In New Zealand the most commonly used acaricides are cyhexatin (withdrawn August 1987), azocyclotin, propargite, bromopropylate and dicofol (Chapman, 1986). These act mainly against the active stages of TSM, although dicofol and bromopropylate have some ovicidal activity.

The use of ovicides to control spider mites is not a new concept. Indeed, petroleum oils (which are ovicidal in action) were some of the earliest acaricides used (Smith and Salkeld, 1966). Interest in the use of ovicidal acaricides has increased in recent times with the introduction of chemicals such as cycloprate and clofentezine which have known ovicidal activity (Asano and Kamei, 1982; Read, 1983).

Hexythiazox is another new acaricide active against the eggs and immature stages of TSM and other tetranychid species (Anon, 1984; Hoy and Ouyang, 1986). At present there is little information about the action of this chemical. The objective of this study was to investigate aspects of hexythiazox toxicity to TSM. In particular, aims of this work were:

(1) to determine the toxicity of hexythiazox to adult female TSM.

(2) to examine the sterilising effect of hexythiazox on adult female TSM.

(3) to establish baseline toxicity levels of hexythiazox to TSM eggs by direct spray and residue exposure bioassay methods.

(4) to examine the effect of egg age, temperature and leaf type on toxicity of hexythiazox to TSM eggs.

(5) to investigate the development of TSM embryos treated with a lethal dose of hexythiazox.

CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

This review has been divided into three sections. Firstly the biology of TSM is discussed; this has been restricted to reproductive biology, and specifically the biology, structure and development of the egg.

The second section covers chemical control of TSM and allied species in general, while examining in detail the use of ovicides and factors affecting their efficacy.

Finally, bioassay methods used for determining the efficacy of ovicides are reviewed. Included in this section is the statistical analysis of bioassay data.

2.2 BIOLOGY OF TSM AND OTHER TETRANYCHIDS

A vast body of knowledge exists on the biology of tetranychid mites, much of which has been covered in a number of review papers. Blauvelt (1945) described the internal morphology of TSM, while Cagle (1949) gave an account of the species' life history. The general biology, ecology, pest status and host-plant relations of tetranychids were reviewed by van de Vrie *et al.* (1972). The ecology of tetranychids and the importance of their natural enemies were discussed by Huffaker *et al.* (1969, 1970) and McMurtry *et al.* (1970). A comprehensive review has recently been produced by Helle and Sabelis (1985); this covers subjects such as anatomy, phylogeny, systematics, reproduction and development, physiology, genetics, ecology, natural enemies and control of tetranychids.

2.2.1 Development of the Egg

2.2.1.1 Oogenesis and Vitellogenesis

Weyda (1980) described the course of oogenesis in TSM as follows: primary cells move from the germarium to the periphery of the ovary and gradually increase in size. Each has a large globular nucleus with one enormous nucleolus, in which little vacuoles and granular parts of two.

types are evident. The cytoplasm contains a great number of free ribosomes, rough endoplasmic reticulum and small globular mitochondria. The primary cells are surrounded by follicular cells, which have elongated nuclei, containing permanently distinct chromosomes. The follicular cells divide frequently. At the periphery of the ovary, the oocytes and nutritive cells differentiate from the primary cells. The surface of the young oocyte is covered with a perforated shell, which is probably formed by the follicular cells. The oocyte increases its size and penetrates into the peripheral pouch where vitellogenesis takes place.

The ovary of TSM is meroistic, i.e., an oocyte-nurse cell complex exists (Feiertag-Koppen and Pijnacker, 1985). The previtellogenic oocytes receive nutrition in the form of proteins and ribosomal RNA via their attached nurse cells. During vitellogenesis yolk precursors, which are synthesised by the midgut epithelium, are taken up by the oocytes. This is made possible by the connection of the oocytes to the midgut cells. The mature oocyte loses its connection with the nurse cells, leaves the peripheral pouch and reaches the posterior cavity of the ovary. The oocyte then passes through the anterior and posterior oviducts and is finally oviposted via the vagina.

2.2.1.2 Embryogenesis

The embryogenesis of TSM has been studied in detail by Dittrich (1968, 1969) and Hurkova and Matolin (1975). The following description is based largely on this work and Crooker's (1985) review.

A newly laid TSM egg is transparent, with visible yolk granules. About 2.5 hours after oviposition (at 22°C) the first cleavage occurs (Dittrich, 1969). Cleavage is complete, leaving two blastomeres of equal size. The second cleavage, which occurs 30 minutes later, is also total thus dividing the egg into four quadrants. Before the third cleavage (about 3.5 h after oviposition) the blastomeres assume a peripheral position. Thereafter cleavages up to the completed blastoderm stage are superficial (Dittrich, 1969). A complete blastoderm is formed by the tenth cleavage, about seven hours after oviposition.

Germ band formation is the next distinct developmental stage. Between the apical parts of the primordial embryo a median furrow

develops. During further development the extremities stretch until the distal parts are parallel to each other. By that time the median furrow has disappeared (Dittrich, 1968).

The germ band grows in all directions around the periphery of the egg until the anterior and posterior parts almost touch. The head lobe develops while the embryo changes from a wormlike to a short and compact shape. Gastrulation and formation of the inner embryonic layer can be observed 24 hours later, following which segmentation of the embryo begins after 30 hours of development (Hurkova and Matolin, 1975). A pair of cheliceri, a pair of pedipalps and four pairs of legs can be distinguished during further growth of the appendages. Subsequently, mouthparts begin to differentiate while growth of the fourth pair of legs is retarded. After 48 hours of development the embryo contracts, the eye spots begin to appear and the appendages continue to differentiate (Hurkova and Matolin, 1975). By 72 hours the embryo has a well developed rostrum, development of the mouthparts is complete and the fourth pair of legs has disappeared. The legs assume their definitive position and pigmentation appears. Finally, after 96 hours air enters the space between the embryo and egg shell, rendering the egg opaque. The surface of the egg becomes wrinkled and soon the six-legged larva emerges (Crooker, 1985).

2.2.2 Formation and Structure of the Egg Shell

Mothes and Seitz (1982) reported that the TSM egg shell is deposited in two phases during vitellogenesis. The first phase occurs at the beginning of vitellogenesis. Small vesicles, seen in the periphery of oocytes, release their contents to form an electron dense layer about 0.3 µm thick, which is interspersed with pores. During yolk uptake no changes to the egg shell occur. However, at the end of vitellogenesis the pores close and the shell thickness increases to 0.5 µm, apparently due to further depositions from vesicles similar to those observed in the first phase.

Little is known of the structure of the TSM egg shell. Studies of related species have been made by Beament (1951) and Lees (1961). Beament (1951), in his study of the European red mite (*Panonychus ulmi*) egg shell, described three shell layers: an outer wax layer, a cement layer and an inner shell layer. The outer layer of the egg shell is composed of wax of a very high melting point, about 1 µm thick. Underlying the

outer wax layer is the cement layer. This is usually around 1 µm thick, is soft and pliable and consists of a mixture of protein and oil. The function of the cement layer is to affix the egg to the substrate on which it is laid. Unlike the former two layers, the inner shell layer completely surrounds the contents of the egg. It is a thin, transparent lamina (less than 0.5 µm thick) and is exceedingly tough and flexible, though not at all elastic. Beament (1951) found a second wax layer was deposited on the inside of the egg following oviposition.

Lees' (1961) study of the structure of the shell of *Petrobia latens* showed it to be very similar to that of *P. ulmi* as described by Beament (1951). The egg shell of *P. latens* is covered in a layer of hard wax. Unlike *P. ulmi*, this layer completely covers the egg. The presence of the cement layer found in *P. ulmi* was not confirmed by Lees (1961). Following the removal of the wax layer a "wrinkled membrane" surrounding the egg shell was observed, but it was uncertain whether this was a homologue of Beament's cement layer in *P. ulmi*. An inner shell layer was also noted and thought to be composed of lipoprotein.

TSM eggs are about 130-140 µm in diameter, smooth-surfaced and perfectly spherical. Although little is known of the structure of the TSM egg shell it is apparently similar to that of related species. Hopp (1954) observed cement and wax layers similar to those of *P. ulmi* described by Beament (1951). Weyda (1980) noted the presence of an hygroscopic secretion, probably glycoprotein, over the egg which is deposited as the egg passes through the anterior and posterior oviducts. In contrast, Mothes and Seitz (1982) found that the egg shell was deposited during vitellogenesis (i.e., while in the ovary).

Mothes and Seitz (1981) determined that the egg shell of TSM consists of three layers: an outer granular layer, a middle dense layer and an inner electron transparent layer. Conversely, Dittrich (1971) described a structureless shell layer about 0.1 µm thick. However, it was assumed that the cement and wax layers were dissolved during preparation for microscopy.

2.2.2.1 The TSM Egg Respiratory Mechanism

Dittrich and Streibert (1969) and Dittrich (1971) described the presence of a respiratory mechanism in the egg of TSM.

The maturing egg develops an air-filled duct system consisting of one frontal and two lateral branches. These ducts are kept open by a multitude of micropillars which expand between the shell and the underlying intermediate lamella. The micropillars arise in the later part of development of the embryo (after 48 hours, 28°C). Their length varies between 0.1 and 0.45 µm.

After about 68 hours of development two perforation organs penetrate the egg shell allowing air to enter the system. These can be seen as brightly contrasting lines on the egg surface (Dittrich, 1968). The perforation organs serve to conduct the respiratory gases from the orifices in the shell through their connected chambers and into the air duct system.

2.2.3 Rate and Duration of Oviposition

The pattern of oviposition in tetranychids generally consists of a short preoviposition period, a rapid increase to a peak rate a few days later, followed by either a slow or a rapid decline (van de Vrie *et al.*, 1972).

The length of the preoviposition period can range from 12 hours (van de Vrie *et al.*, 1972) to as much as eight days (Cagle, 1949). This is, however, largely dependent on temperature. Around one day appears typical for TSM (van de Vrie *et al.*, 1972).

Egg production also appears to be dependent on temperature. van de Vrie *et al.* (1972) considered five to six eggs per female per day to be average for many tetranychid species. Cagle (1949) obtained a range of 2.1 to 7.5 eggs per female per day in his experiments.

More meaningful estimates of egg production may be made by considering total production over the lifetime of a mite. In a number of studies reviewed by van de Vrie *et al.* (1972) average numbers of eggs produced per female TSM ranged from 15.8 to 111, with a maximum of 202. Measures of average total egg production per female TSM by Cagle (1949)

ranged from 39.8 to 100.1.

Egg production, development and survival can be markedly affected by a variety of factors. Important among these are environmental conditions (particularly temperature and humidity), seasonal influences, host-plant interactions and biotic factors (such as population density). Many of these factors have been discussed by van de Vrie *et al.* (1972) and Wrensch (1985).

2.3 OVICIDAL CONTROL OF TSM

2.3.1 Ovicidal Acaricides

For most of this century chemical applications have been the basis of control of TSM and other tetranychid mite pests. This began with the use of sulphur dusts. Petroleum oils and the dinitrophenol compounds came into use in the 1920's and 1930's. A major change in pest control occurred with the development of synthetic organic pesticides following World War II. Since then a wide range of chemical groups have been used as acaricides, including organochlorine, organophosphorous, carbamate, and sulphur compounds. More recently the organotin and synthetic pyrethroid compounds have been used. The rapid rate at which tetranychid mites have developed resistance to acaricides has necessitated the continued development of new chemical groups. Cranham and Helle (1985) listed 19 widely-used groups of acaricidal pesticides. Of these many are active mainly against the adult and immature mite stages, particularly those groups that are primarily insecticidal with secondary acaricidal activity such as the organochlorine, organophosphorous and carbamate groups. However, several acaricides have at least some degree of ovicidal action.

The activity and mode of action of common acaricide groups are summarised in Appendix I.

2.3.2 The Toxicity of Ovicides to Different Developmental Stages

Acaricides commonly regarded as ovicides, such as chlorfenson, tetradifon, cycloprate, clofentezine and hexythiazox are generally toxic to larvae as well as eggs. Toxicity of these chemicals to the nymphal stages is variable and is generally very low to adults.

For example, Ebeling and Pence (1954) found larval TSM to be most susceptible to chlorfenson treatment (LC_{50} 2.8 x 10^{-2} % a.i.); eggs were about three times as tolerant (LC_{50} 1.1 x 10^{-1} % a.i.) while adults were more than 150 times as tolerant (LC_{50} 4.25 % a.i.). Similarly, Asano and Kamei (1977) found larvae of the citrus red mite (*Panonychus citri*) to be most susceptible to cycloprate (LC_{50} 6.0 x 10^{-3} % a.i.) followed by eggs (LC_{50} 1.4 x 10^{-2} % a.i.) and protonymphs (LC_{50} 2.5 x 10^{-2}% a.i.). The deutonymphs (LC_{50} 1.2 x 10^{-1} % a.i.) and adults (LC_{50} > 4.0 x 10^{-1} % a.i.) were of low susceptibility. Aveyard *et al.* (1986) compared the toxicity of clofentezine to different stages of TSM. Eggs were found to be most susceptible (LC_{50} 1.6 x 10^{-5} % a.i.), then larvae (LC_{50} 6.2 x 10^{-4} % a.i.) and protonymphs (LC_{50} 1.0 x 10^{-3} % a.i.). Deutonymphs and adults were relatively less susceptible (both LC_{50} > 1.0 x 10^{-2} % a.i.). Another study (Anon., 1984) showed hexythiazox to be highly toxic to eggs and immature stages of TSM (LC_{50} egg 3.4 x 10^{-5}, larva 2.3 x 10^{-5}, protonymph 2.8 x 10^{-5} and deutonymph 3.0 x 10^{-5} % a.i.) but of low toxicity to adults (LC_{50} 5.0 x 10^{-2} % a.i.).

Although that study (Anon., 1984) gave LC_{50} values for the larval and nymphal stages, Chapman (1986 and pers. comm.) found that these stages are not directly killed; treated immatures developed through to the subsequent chrysalis stage but failed to emerge. The same phenomenon was found to occur in immature stages of the citrus red mite (*Panonychus citri*) treated with cycloprate (Asano and Kamei, 1977).

An additional factor in the action of some ovicides is their activity as chemosterilants. Tetradifon (Batth and Davidson, 1959), cycloprate (Asano and Kamei, 1977) and clofentezine (Chapman and Marris, 1986) have been shown to have chemosterilant effects. Typically female mites treated with these chemicals continue to lay eggs, although often at a reduced rate (Asano and Kamei, 1977; Chapman and Marris, 1986), but the eggs fail to hatch.

2.3.3 Factors Affecting the Efficacy of Ovicides

The efficacy of pesticides can be influenced by a variety of factors. These can be separated into biological, environmental and operational factors. These factors will be discussed as they affect ovicidal control of tetranychid mite eggs, particularly those of TSM.

2.3.3.1 Biological Factors

Egg Age:

The toxicity of several ovicides has been shown to be affected by egg age. Harrison and Smith (1961) investigated this factor for five ovicides against TSM. The compounds were: chlorbenside, chlorfenson, Supona, tetradifon (all sulphur-based ovicides) and dicofol (a bridged diphenyl ovicide). There was a marked decrease in toxicity to eggs treated with the sulphur-based ovicides. Over an egg age range of 0 to 72 hours susceptibility decreased by 5.9 times for Supona, 40 times for chlorbenside, 170 times for tetradifon and 460 times for chlorfenson. However, the susceptibility of eggs treated with dicofol did not vary with age. Harrison and Smith (1961) also noted that, in eggs treated with these ovicides immediately prior to hatch, the amount of ovicide required to obtain a reasonable kill was so great that eggs at this stage of development are virtually immune to these chemicals.

Staal *et al.* (1975) found that the susceptibility of TSM eggs treated with cycloprate did not change significantly with age. However, a study by Asano and Kamei (1978) using cycloprate treated eggs of the Kanzawa spider mite (*Tetranychus kanzawai*) showed a decrease in susceptibility with age. LC_{50} values were 0-24 hours (9.7×10^{-4} % a.i.), 24-48 hours (1.29×10^{-3} % a.i.), 48-72 hours (1.23×10^{-3} % a.i.), 72-96 hours (4.15×10^{-3} % a.i.) and 96-120 hours (>5.0×10^{-3} % a.i.).

Aveyard *et al.* (1986) and Neal *et al.* (1986) reported a decrease in susceptibility of TSM eggs treated with clofentezine with age. Hoy and Ouyang (1986) found hexythiazox-treated eggs of the Pacific spider mite (*Tetranychus pacificus*) became less susceptible with age. Likewise, Welty *et al.* (1987) found that susceptibility of European red mite (*Panonychus ulmi*) eggs treated with hexythiazox decreased with age.

For the above examples, with the exception of dicofol (Harrison and Smith, 1961), a trend towards decreased susceptibility with age has been shown. The reason for this is unclear. Aveyard *et al.* (1986) suggested that the embryo may become less susceptible or, alternatively, penetration through the chorion may occur slowly, thus preventing sufficient active ingredient from reaching the embryo with later applications. In support of the latter view, Hopp (1954) found that of

three ovicides (clorobenzilate, p-chlorophenyl sulphonate and chlorfenson), chlorobenzilate was found to kill the embryo at an earlier stage. This difference was attributed to rate of penetration through the chorion, since all three ovicides were equally toxic to embryos removed from the chorion. Moreover, the high toxicity of many ovicides to the larval stage suggests that the embryo does not increase in tolerance with age.

Egg Type:

Some tetranychid mite species such as the European red mite (*Panonychus ulmi*), *Petrobia latens*, *P. apicalis* and *Bryobia rubrioculus*, overwinter in the egg stage. Studies of overwintering mite eggs have revealed that their structure differs from summer forms (Beament, 1951; Lees, 1961). This is reflected in the toxicity of hexythiazox to the different egg types. Welty *et al.* (1987) found that *P. ulmi* summer eggs (LC_{50} 2.2 x 10^{-4} % a.i.) were approximately nine times more susceptible than winter eggs (LC_{50} 2.0 x 10^{-3} % a.i.). Aveyard *et al.* (1986) found clofentezine to be toxic to both winter and summer eggs of *P. ulmi*, although the data were not sufficient to indicate any differential toxicity. However, they noted that amitraz, benzoximate and fenazoflor were toxic to *P. ulmi* summer eggs but were inactive against winter eggs.

Proportion of the Population in the Egg Stage:

Smith and Salkeld (1966) considered that, for the egg stage to be a practical target for ovicidal control, it must represent a significant segment of the population. This has been shown to be the case for TSM.

Using a simulation model Carey (1982) showed that shortly after growth begins in a mite population, (e.g., the development of the first generation of a growing season) eggs become the predominant life history stage. Subsequent hatch of these eggs produces a preponderance of immatures. However, this wave does not continue to the adult stage because adult females have an extremely high egg deposition rate shortly after becoming sexually mature. Therefore their contribution of eggs quickly swamps the contribution they themselves make to the life stage structure of the population. Thus in a growing mite population eggs make up a large proportion of the population.

Moreover, once Carey's (1982) simulated population reached a stable level the egg stage remained a significant proportion of the population (about 65%). Butcher (1986) studied the population structure of TSM on strawberry plants, and found that eggs constituted 32% of the total population on first-year plants, averaged over the whole season. On second-year plants the proportion was 61%, and 52% on third-year plants. Studies reviewed by Carey (1982) of TSM populations on a variety of host plants showed that eggs account for 56-69% of the total population.

This suggests that with respect to demography, TSM would be a suitable target for ovicidal control.

Location of the Egg:

For an ovicide to be effective eggs must be in an exposed location where lethal concentrations of toxicant can be directed to it (Smith and Salkeld, 1966).

TSM eggs are predominantly laid on the undersides of leaves, which is where most TSM adults are found. Some eggs may be laid on the upper surface of the leaf, but in the mite colonies used in this study this occurred more at higher population densities. Few eggs are laid on leaf stems. Beament (1951) noted that summer eggs of *P. ulmi* tend to be laid in pits or depressions on the leaf surface or in the crevices formed by leaf veins. Experiments conducted by Beament (1951) suggested that oviposition sites of high humidity were favoured. The same appears to be true for TSM.

As TSM eggs are found generally in the same areas as motile forms, difficulties encountered in obtaining adequate spray coverage with acaricides active against motile forms are also likely to occur with ovicidal sprays.

Host-plant Effects:

Asano and Kamei (1982) found that the toxicity of cycloprate to eggs of several tetranychid species varied with the test host plants. For example, TSM eggs on grape and peach leaves were more than three times more susceptible than those on soybean leaves. Further experiments on upper and lower leaf surfaces showed that eggs on the upper surface of apple leaves were about three times more susceptible

than eggs on the underside. However, there was no difference in the toxicity of cycloprate between peach leaf surfaces. Asano and Kamei (1982) considered the toxicity differences to be due in part to the fine structure and physiological characteristics of the leaf surface.

Wakou and Sugawara (1974) compared the toxicity of dicofol to TSM eggs on three leaf types: peach, bean and apple. The responses of the eggs differed with the exposure method used; leaf dip and spray. At low spray volumes eggs laid on peach leaves were most susceptible, followed by bean and apple. Using the leaf dip method, eggs laid on apple leaves were most susceptible followed by bean and peach. The toxicity differences were thought to be due to differences of the surface structure of the leaves and the amount of chemical deposition.

Stevens and Baker (1987) investigated the foliar absorption and redistribution of herbicide sprays on a variety of leaf types. Differences in foliar uptake and spread were noted between the different leaf types. Similar properties of ovicides may account for differential toxicities between leaf types.

2.3.3.2 Environmental Factors

Temperature:

Several studies have shown that the toxicity of a compound can be affected by temperature. For example, temperature and toxicity are positively correlated for most organophosphorous and carbamate insecticides, while synthetic pyrethroid insecticides typically have a negative temperature-toxicity correlation (Sparks *et al.*, 1982).

Temperature has been shown to be a significant factor in the action of some ovicides. Neal *et al.* (1986) found that the toxicity of clofentezine and cycloprate to eggs of TSM and the carmine spider mite *(Tetranychus cinnibarinus)* was higher at 16°C than at 22°C. Stenseth (1976) showed that by increasing temperature from 15°C to 27°C the toxicity of quinomethionate to adult TSM increased slightly, but ovicidal activity was reduced. The toxicity of cyhexatin to TSM adults and eggs was positively correlated with temperature, but dicofol toxicity did not change. Harrison and Smith (1961) found that there was no difference in the toxicity of dicofol, tetradifon and chlorbenside over a temperature range of 16°C to 29.5°C.

Humidity:

The influence of humidity on the toxic action of ovicides is not well documented. Harrison and Smith (1961) examined the toxicity of five ovicides; dicofol, chlorbenside, chlorfenson, Supona and tetradifon, to TSM eggs over a range of relative humidities (30, 50, 70, 90 and 96% R.H.). A positive humidity-toxicity correlation was found. Over the humidity range used a 14-fold increase in toxicity occurred for chlorbenside, 112-fold for dicofol, 125-fold for Supona, 916-fold for tetradifon and 2000-fold for chlorfenson.

Beament (1951) noted that *Panonychus ulmi* eggs tend to be laid in pits and depressions on leaves, apparently because of higher humidity. If, as Harrison and Smith (1961) found, ovicides tend to be more toxic in more humid conditions this oviposition behaviour would appear to enhance toxicity.

2.3.3.3 Operational Factors

Formulation:

Ebeling and Pence (1954) compared wettable powder (WP) and emulsifiable concentrate (EC) formulations of 10 acaricides against TSM eggs, larvae and adults. Of these, the EC formulations were generally most effective. For example, the LC_{50} for TSM eggs treated with chlorobenzilate WP formulation was 1.26×10^{-1} % ai and for the EC formulation 7.8×10^{-2} % ai.

Neal *et al.* (1986) investigated the effect of clofentezine formulation on toxicity to TSM eggs. Two WP formulations (mass mean particle diameter 3.0 µm) and a soluble concentrate (SC) formulation (mass mean particle diameter 1.9 µm) were used. The SC formulation, with the smaller particle size, was significantly more toxic than the two WP formulations, which were of equal toxicity.

Ebeling and Pence (1954) noted that the relative initial effectiveness of an acaricide may not be the principal factor affecting long-term control efficiency in the field. For example, of seven acaricides that were applied to avocado trees, in all cases the WP formulations remained effective against adult TSM longer than EC formulations.

Spray Coverage:

The egg as a target site differs significantly from motile stages because of its immobility and small size (130-140 µm in diameter).

Munthali and Scopes (1982) and Munthali (1984) investigated the relationship between dicofol droplet distribution and size, and toxicity to TSM eggs. It was noted that at a droplet diameter of 55 µm and at densities of up to 200 droplets cm^{-2}, few eggs (less than 10%) were directly hit. Moreover, no obvious relationship between the number of direct hits and mortality was found. For example, very high mortalities occurred even when all eggs were missed. Thus it was concluded that activity must rely on the spread of dicofol on or through the leaf after deposition of the droplet.

Munthali (1984) found the toxicity of dicofol to be inversely related to droplet size for a given concentration. The following LC_{50} values were calculated for the respective droplet sizes: 20 µm (LC_{50} 12.0 ng cm^{-2}), 40 µm (26.5), 60 µm (44.5), 80 µm (64.5) and 100 µm (86.0). As the effectiveness of a droplet is dependent on the spread of the droplet on the leaf and then on the diffusion of the toxicant from the perimeter, he concluded that success can best be achieved by applying as many droplets to the surface as possible using a formulation capable of giving maximum spread. For a given volume of spray solution, smaller droplet size will result in higher droplet density and therefore less chemical spread is necessary to ensure mortality.

Surfactants may also be a significant factor in spray coverage. Stevens and Baker (1987) investigated the effect of the surfactant Ethylan TU on the spread and absorption of three herbicides using a variety of surfaces. In all cases spread was enhanced by the addition of the surfactant. However, the foliar absorption response was variable. For example, the spread factor (the ratio between the diameter of the dried deposit and that of the in-flight droplet) of glyphosate on apple was more than doubled by the addition of the surfactant. Foliar absorption was virtually unaffected.

If, as suggested by Munthali (1984), the toxicity of a chemical to a stationary object such as a mite egg is dependent on the chemical's spread, then the toxicity of such chemicals may be enhanced by the addition of a surfactant.

2.4 BIOASSAY OF OVICIDES

2.4.1 Bioassay Methods

The most commonly used bioassay method for testing ovicides against spider mite eggs is the leaf disc method, or variations on it (Helle and Overmeer, 1985). This involves placing adult female mites on leaf discs for a period of time (generally 24 hours) over which eggs are laid. Following oviposition the adult female mites are removed and the discs sprayed under a Potter tower (Potter, 1952) or similar device. Leaf discs are maintained on moistened cotton wool which prevents desiccation of the leaf disc and forms a barrier to motile forms such as the females when ovipositing and larvae that may hatch. Leaf discs are held under standard temperature, humidity and light conditions. Mortality is checked several days later depending on the developmental rate of the species being tested. This method is simple, relatively quick and produces repeatable results.

Adaptations of this method include the use of entire leaves rather than leaf discs and, when no spraying device is available, leaf discs may be dipped in the test solution (Helle and Overmeer, 1985). The leaf disc method may also be used to determine residual toxicity by spraying or dipping the discs before eggs are deposited.

To enable environmental conditions, particularly humidity, to be controlled Harrison and Smith (1961) developed a method whereby mite eggs were laid upon microscope slides. Adult female mites were placed in a cage between two microscope slides and left to oviposit. Following the oviposition period the mites were removed leaving eggs on the slides. After treatment, slides were placed in boxes in which humidity and temperature could be regulated. Harrison and Smith (1961) argued that because of complicating factors such as transpiration and radiant heating, environmental conditions on leaves or leaf discs could not be adequately controlled. Conversely, absence of a leaf surface may reduce or negate the action of some ovicides. For example, Munthali and Scopes (1982) showed that transportation of dicofol over or through the leaf surface is an important factor in the toxic action against mites. The method of Harrison and Smith (1961) is considerably more time-consuming than the leaf disc method.

2.4.2 Treatment Methods

Dipping and spraying are the most frequently used treatments for ovicidal bioassays.

Dip methods, as the name implies, involve dipping eggs, which may be on leaf discs, leaves or slides, into a treatment solution, typically for a period of 5 to 10 seconds. Spray methods use some form of spraying device such as the Potter tower (Potter, 1952) to produce an atomized spray which is directed at the test subject.

In favour of the dip method is its simplicity and the lack of specialised equipment required. However, several criticisms of this technique have been made. Suckling (1983) noted that dipping methods may produce deposits widely different from those in the field, particularly for low volume application, where trees are not sprayed to runoff. Variability may also occur because of uneven coverage. Dittrich (1962) found that, using the leaf dip method, the toxicant tended to gather around the midrib, a preferred site of mites.

Suckling (1983) considered spray methods to be superior to dipping because of their greater similarity to field deposits. Moreover, an accurate estimate of spray deposit and distribution may be made (Potter, 1952). While more time-consuming and requiring greater skill (Dittrich, 1962), spray methods allow more accurate dosing (Helle and Overmeer, 1985) and produce less variable results than dip methods (Dittrich, 1962).

2.4.3 Statistical Analysis

Probit analysis has been used extensively for the analysis of toxicological bioassay experiments which yield dose-response type data (Finney, 1971; Hoskins and Craig, 1962).

Plots of dose-response data typically produce assymetric sigmoid curves. These curves can be linearised by the transformation of dosages to logarithms and response to probits. Probits are produced by the conversion of percentage response to standard deviation and the addition of five as a constant (Hoskins and Craig, 1962). The log dosage - probit line is abbreviated as the ld-p line (Hoskins and Craig, 1962).

For toxicological experiments an LD_{50} (lethal dose) or LC_{50} (lethal

concentration) is commonly used. This is the dosage or concentration value at which death (or whatever response is measured) occurs in 50% of the exposed subjects. The slope of the ld-p line expresses the variability in susceptibility of a test population. A steep line indicates that a population is relatively homogeneous in susceptibility and a flatter line indicates a population varying widely in susceptibility (Hoskins and Craig, 1962).

Finney (1977) used the chi-squared test to determine whether the line is an adequate representation of the data within the limits of random variation. A large chi-squared value may indicate that the test subjects do not react independently or that the straight line does not adequately describe the relationship between dosage and mortality. If the probability of a chi-square value is greater than 0.05 the line adequately fits the data. However, if the probability is less than 0.05 there is a systematic departure from the points of the regression.

Finney (1977) gave a thorough account of the statistical methods involved in calculation of the ld-p line. Calculation has been greatly simplified by the development of computer programmes such as POLO (Russell *et al.*, 1977; Robertson *et al.*, 1980).

CHAPTER 3
METHODS AND MATERIALS

3.1 SPIDER MITE STRAIN

A single strain of TSM, designated Auckland 1 (A-1), was used throughout this study. The strain was collected in February 1985 from a commercial rose house producing export grade flowers where the mites had been sprayed with a variety of miticides and insecticides.

Adult female TSM from this strain were tested by Chapman (1986) using the slide-dip method (FAO, 1974) to determine base-line toxicity data for this strain. The results for four chemicals are shown in Table 3.1.

Table 3.1: Toxicity of chemicals to A-1 strain of two- spotted mite using the slide-dip method.

Chemical	LC_{50}*	95% C.L.	Slope	SE
azinphos-methyl	0.34	0.30 - 0.39	2.97	0.29
fenvalerate	4.93	3.01 - 6.04	4.63	0.77
cyhexatin	0.23	0.08 - 0.37	2.82	0.31
propargite	4.95	3.23 - 6.57	1.43	0.03

* LC_{50} expressed in grams a.i. L^{-1}.

Comparing the A-1 strain with a susceptible strain collected from a home garden crop, there was a significant difference ($p < 0.05$) only between LC_{50} values of azinphos-methyl, the A-1 strain being about 3.5 times more tolerant. No chemical selection pressure has been placed on the A-1 strain since the colony was established.

The A-1 strain used in this study was reared on French dwarf bean,

Phaseolus vulgaris (cv. 'Tendergreen') and was maintained in a controlled-temperature room at 24 ± 3°C, 50-80% relative humidity and a 16L:8D photoperiod.

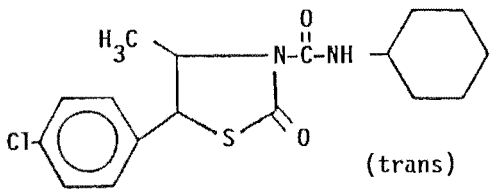
Host plants were grown in 15 cm pots in a glasshouse. When plants were sufficiently developed they were placed among already colonised plants. Mites from established bean plants migrated onto new plants. As mite numbers built up host plants became chlorotic and were periodically removed.

Contamination from other strains was avoided by placing the host plant pots in water trays. Other mite colonies were kept in separate rooms to minimise the risk of aerial migration. Unnecessary movement between rooms was avoided to reduce the risk of human transmission of mites.

3.2 CHEMICAL AND PHYSICAL PROPERTIES OF HEXYTHIAZOX

Hexythiazox is an acaricidal compound of thiazolidinone structure (Anon., 1984).

Common name : hexythiazox
Trade name : Nissorun (in New Zealand)
Chemical name : trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidinonecarboxamnide
Structural formula:



(trans)

Empirical formula : $C_{17}H_{21}ClN_2O_2S$
Molecular weight : 352.5
Appearance : white crystal

Odour	:	odourless
Melting point	:	105.5°C
Solubility (20°C)	:	chloroform 137.9 (g.100 ml^{-1}), acetone 16.0, n-hexane 0.39, methanol 2.06, xylene 36.2, acetonitrite 2.86, water 0.5 (ppm)
Vapour pressure	:	2.54×10^{-8} mmHg (20°C)

A 10% wettable powder formulation was used throughout this study. Suspensions for toxicological tests were made up in distilled water.

3.3 THE TOXICITY OF HEXYTHIAZOX TO ADULT FEMALE TSM

The toxicity of hexythiazox to adult female TSM was determined using the slide-dip method (FAO, 1974). A piece of double-sided ScotchTM tape was placed on a microscope slide. Twenty TSM were transferred to the tape using a moistened camel hair brush (size 000) and affixed by the dorsal part of the hysterosoma. Slides were dipped in a given aqueous suspension of hexythiazox for five seconds. After dipping the slides were left to drain on filter paper; any excess was carefully blotted off.

After treatment the slides were placed in a covered plastic trays lined with moistened filter paper. The trays were then placed in a controlled temperature cabinet (Contherm Precision Cabinets Ltd, Lower Hutt, New Zealand) which was maintained at 25 ± 0.5°C, 70-80% relative humidity and a 16L:8D photoperiod.

Mortality was assessed following a 24 hour post-treatment period. Mites were considered dead if they could not move their legs after gentle prodding with a fine camel hair brush.

3.4 SPRAY TOWER EXPERIMENTS

The following experiments involved spray applications using a Potter tower (Burkhard Manufacturing Co. Ltd, Rickmansworth, U.K.).

3.4.1 Calibration of the Potter Tower

The Potter tower was calibrated to determine the quantity of residue deposited and to detect any change in the deposit level throughout testing.

Initially distilled water was used for calibration, but because the high rate of evaporation precluded accurate weighing of the deposit, mineral oil was used.

Two ml mineral oil (Shell Supreme Extra All Purpose Spraying Oil) was sprayed by the Potter tower (at 103 ± 10 kPa, with 10 seconds settling time) onto microscope slide coverslips (diameter 22 mm). The coverslips were weighed using a Sartorius 1712 MP 8 balance (± 0.02 mg) and the net mineral oil deposit was calculated.

Five coverslips were used per treatment. One was placed in the centre of the spray platform, and the other four were positioned 3.7 cm to the back, front, left and right of the centre coverslip. Thus an indication of the spray distribution was also obtained.

3.4.2 Alterations to the Potter Tower

In the latter part of this study modifications were made to the Potter tower so that the platform could be raised independent of the delivery of spray. Previously the platform was raised by the same air flow as used for the atomizing spray. This resulted in a varied air pressure during spraying which caused variability in droplet size. The modified system produced a nearly constant spray pressure resulting in more even droplet size (Manktelow, pers. comm.).

The leaf type experiment (3.6.4) was conducted after these modifications. Changes in spray deposit due to these modifications mean that no direct comparisons can be made between the leaf type experiment and prior experiments.

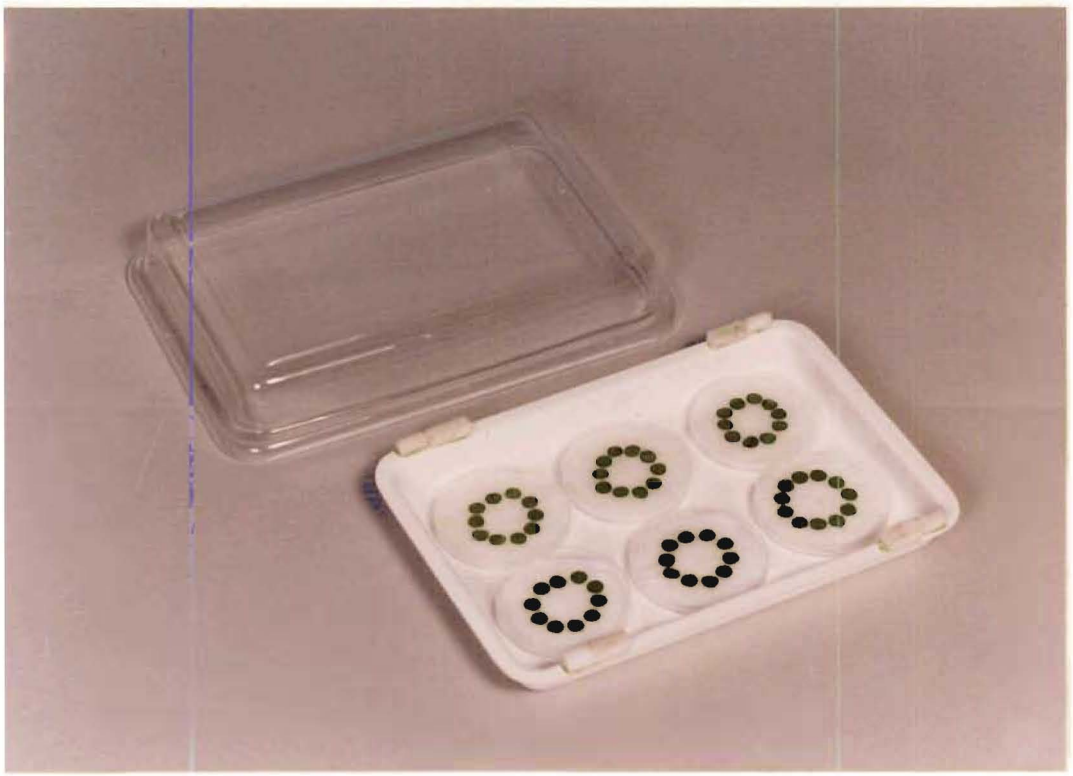
3.4.3 The Leaf-Disc Method

A modification of the leaf-disc method described by Overmeer and van Zon (1973) was used for all the following experiments.

To obtain adequate numbers of TSM eggs, a series of petri dishes were set up containing moistened cotton wool on which ten broad bean *(Vicia faba)* leaf discs (10 mm diameter) were placed upside down (see Plate 1). Ten to 15 adult female TSM were transferred onto each leaf disc using a moistened camel hair brush. The petri dishes were placed in covered plastic trays (23 x 33 cm) (see Plate 2). Sufficient water to

PLATE 1: Petri dish and broad bean leaf discs used for ovicide bioassay experiments

PLATE 2: Plastic tray used to hold petri dishes for bioassay experiments



cover the base of the trays was added to ensure a humid atmosphere within the tray. Blocks of closed cell foam (10 mm thick) were glued at each corner of the trays to raise the lids and thus prevent the atmosphere from becoming saturated. Holes in the base of the petri dishes allowed water from the tray to enter to keep the cotton wool moist and the leaf discs fresh.

Trays were placed in a controlled temperature cabinet maintained at 25 ± 0.5°C, 70-80% relative humidity and 16L:8D photoperiod.

After 24 hours the mites were removed from the leaf discs either by brush or by suction (using a fine pipette tip attached to a vacuum pump by a plastic hose). The latter method was found to be as effective as brush removal and considerably faster.

Individual petri dishes were then sprayed under a Potter tower at a pressure of 103 ± 10 kPa, with a settling time of 10 seconds. A 1.5 ml spray volume was used, resulting in a uniform coverage of droplets on the leaf surface with no significant runoff. Controls were sprayed with 1.5 ml distilled water. Spraying was done at room temperature (18-25°C). Prior to spraying the Potter tower was washed out with commercial grade acetone followed by distilled water.

Following treatment the trays were left uncovered for half an hour to allow the leaf surfaces to dry and were then covered and returned to the controlled temperature cabinet. During incubation checks were made on the hatch of untreated eggs of the same age. Once no change in the hatch of untreated eggs was noted, the mortality of treated eggs was checked. Checks were made periodically to ensure no further hatch occurred.

Mortality was assessed as non-hatch of the eggs. Larvae which pierced the egg shell but failed to emerge entirely were recorded as dead. The survival of hatched larvae was not recorded.

3.5 THE STERILISING EFFECT OF HEXYTHIAZOX

A modified leaf disc method was used to examine the sterilising properties of hexythiazox on adult females. Unless stated otherwise the same methodology described in 3.4.3 was used.

3.5.1 The Effect of Direct Exposure on Adult Female TSM

Recently mated female TSM were transferred to individual leaf discs on moistened cotton wool in petri dishes. Twenty mites were used per treatment.

Leaf discs were sprayed under the Potter tower with 1.5 ml hexythiazox solution at the suggested field rate (2.5×10^{-3}% a.i.) and one-tenth of the field rate (2.5×10^{-4}% a.i.).

One hour after spraying each mite was placed on a separate residue-free leaf disc. Every two days females were further transferred to fresh discs; this was continued for 21 days. The number of eggs laid and their mortality was recorded.

3.5.2 The Effect of Length of Exposure of Adult Female TSM to Residues

Leaf discs were sprayed with the suggested field rate concentration of hexythiazox under the Potter tower. Recently-mated female TSM were placed onto individual leaf discs. Twenty mites were used per treatment.

Mites were left on the sprayed leaf discs for 1, 3 or 5 days following which they were transferred to residue free leaf discs. They were subsequently transferred to fresh leaf discs every two days for a total of 12 days.

The number of eggs laid and their mortality was recorded.

3.6 OVICIDAL TOXICITY TESTS

3.6.1 The Effect of Exposure of Eggs to Residues

The standard leaf-disc method as described in 3.4.3 was used, except that female TSM were placed on the discs after they had been sprayed with hexythiazox at a range of concentrations. The mites were left to oviposit for 24 hours and then removed, so that eggs were laid onto a residue of hexythiazox.

3.6.2 The Effect of Post-Treatment Temperature on Toxicity

Again the standard leaf-disc method was used, but following treatment at a range of concentrations trays were incubated in controlled temperature cabinets at 15, 20, 25 or 30°C.

3.6.3 The Effect of Egg Age on Toxicity

The toxicity of hexythiazox to eggs of six age groups: 0-6, 6-12, 12-24, 24-48, 48-72 and 72-96 hours, was examined.

For the 0-6 and 6-12 hour age groups the female TSM oviposited for six hours. The 0-6 hour age group eggs were sprayed immediately after removal of the females, while the 6-12 hour age group were sprayed six hours later.

A 12-hour oviposition period for the 12-24 hour age group was used. Eggs were sprayed 12 hours after removal of the females.

A 24 hour oviposition period was used for the 24-48, 48-72 and 72-96 hour age groups. Eggs were sprayed after 24, 48 and 72 hours respectively following removal of the females.

Incubation of all treatments was at 25°C.

3.6.4 The Effect of Leaf Type on Toxicity

In addition to broad bean, four other leaf substrates were used to determine their effect on toxicity. The leaf types used were: broad bean (*Vicia faba*, cv. 'Exhibition Long Pod'), strawberry (*Fragaria X ananassa*, cv. 'Red Gauntlet'), raspberry (*Rubus idaeus*, cv. 'Glen Prosen') and two apple cultivars (*Malus*, sp. cv. 'Red Delicious' and cv. 'Granny Smith').

To obtain information on the variations in lamina surface morphology of the five species, samples were prepared for examination by scanning electron microscope (SEM) (Cambridge 250 MK. 2). Leaf discs were cut and adult female TSM allowed to oviposit on them for six hours at 30°C. The eggs were then incubated for 18 hours at 25°C, following which the leaf discs were placed on filter paper for four hours to absorb extraneous water. The leaf discs were then gold-coated and viewed under the SEM.

3.7 EXAMINATION OF THE DEVELOPMENT OF TREATED TSM EGGS

3.7.1 Electron Microscopy

For comparisons of treated and untreated eggs the eggs were sprayed under the Potter tower with 1.5 ml hexythiazox at 1 x 10^{-4}% a.i.

(approximately the LC_{90} value) and 1.5 ml distilled water respectively. The eggs were incubated at 25°C until no further nontreated eggs hatched.

Eggs were air-dried by placing leaf discs on filter paper for four hours prior to gold-coating. The eggs were then viewed under the SEM. Initial attempts at freeze-drying the eggs were unsuccessful; most eggs collapsed when placed under vacuum in the SEM. Air-drying was found to be simpler and more effective, with only a few eggs collapsing.

3.7.2 Light microscopy

TSM eggs were examined using a Reichert Diapan microscope with a 10x objective lens magnification and 16x eyepiece. Photographs were taken on a Nikon Microflex model EFM microscope attachment using Ilford Pan F (50 ISO) film.

Adult female TSM were placed on broad bean leaf discs and allowed to oviposit for four hours at 30°C. After removal of the females the eggs were sprayed under the Potter tower with 1.5 ml hexythiazox at 1 x 10^{-4}% a.i. (approximately the LC_{90} value). Controls were treated with 1.5 ml distilled water. The eggs were incubated at 25°C.

Checks were made on control eggs to determine the time at which most had hatched. Following this, treated eggs were prepared on microscope slides. Two adhesive plastic ring eyelets (Quickstik^{R}) were placed on top of each other on a microscope slide to form a cavity. Treated eggs were removed from leaf discs by a moistened camel hair brush and placed in the slide cavity. A drop of Heinz mounting medium was pipetted into the cavity and a coverslip placed on top. The slides were then heated briefly by passing them over a bunsen burner until the eggs became transparent. The slides were then ready for inspection under the microscope.

3.8 ANALYSIS OF RESULTS

Probit analysis was used to analyse dose-mortality data. Log-probit lines, LC_{50} values and their 95% confidence limits were calculated using the POLO computer programme (Robertston *et al.* 1980). Experiments were replicated until the G(0.95) value, the index of significance for potency estimation, was below 0.4. Significant differences in LC_{50} values were determined by non-overlap of 95% confidence intervals.

CHAPTER 4
RESULTS

4.1 INTRODUCTION

Results are given in the same order as outlined in the Methods and Materials section.

Dosage-mortality test results (with the exception of the sterilising effect experiments, 4.3.2) are given as a tabulated summary from the log-probit analysis produced from the POLO programme. Pooled data used in the analyses are given in Appendix II.

It was necessary to omit some data points for the production of log dose-probit graphs, but these points were included in the probit analyses.

4.2 THE TOXICITY OF HEXYTHIAZOX TO ADULT FEMALE TSM

The toxicity of hexythiazox to adult female TSM using the slide-dip test is summarised in Table 4.1.

Table 4.1: Toxicity of hexythiazox to adult female twospotted spider mite using the slide-dip technique.

LC_{50}*	95% C.L.	Slope	SE
1.48	1.37 - 1.57	8.18	1.62

* LC_{50} expressed in grams a.i. L^{-1}.

The LC_{50} concentration is 59 times greater than the suggested field concentration (0.025 g a.i. L^{-1}). Therefore, for all practical purposes, hexythiazox can be considered non-toxic to adult female TSM.

4.3 SPRAY TOWER EXPERIMENTS

4.3.1 Calibration of the Potter Tower

Calibration of the Potter tower by weighing deposits of mineral oil sprayed onto coverslips produced an average of 1.36×10^{-3} g cm^{-2} before any experimentation. However, following the series experiments up to when the Potter tower was modified, an average deposit of 1.17×10^{-3} g cm^{-2} was calculated. A two-tailed t-test showed the deposit levels to be significantly different ($p < 0.01$).

The Potter tower was again calibrated following the modifications outlined in 3.4.2. An average deposit of 9.24×10^{-4} g cm^{-2} was calculated. A two-tailed t-test showed this deposit to be significantly lower than either of the previous calibrations ($p < 0.001$).

Analysis of droplet size was made following the modifications using the magnesium oxide method (May, 1950). A volume mean droplet diameter of 42 µm was calculated (Manktelow, unpublished data).

4.3.2 The Sterilising Effect of Hexythiazox

4.3.2.1 The Effect of Direct Exposure on Adult Female TSM

The effects on egg laying and hatching of female TSM exposed to direct sprays of hexythiazox are summarised in Table 4.2.

Table 4.2: The effect of direct sprays of hexythiazox on egg laying and hatching of twospotted mite.

Concentration*	Mean eggs laid/ female/day	SD	Mean eggs hatched/day	SD	Percentage hatch
Field rate (2.5)	2.41	0.99	0.82	0.78	34.0
One tenth field rate (0.25)	2.41	1.07	1.42	0.86	58.9
Untreated control	3.42	0.98	2.81	0.84	82.2
LSD (0.05)	0.66		0.49		

* Concentration expressed as % a.i. $\times 10^{-3}$

Using the LSD test oviposition was found to be significantly ($p < 0.05$) reduced at both field rate and one tenth field rate concentrations compared with untreated females. Moreover, the hatch rate was significantly ($p < 0.05$) lowered at both concentrations although this was most pronounced at the field rate.

Figure 4.1 shows the change in percentage hatch following direct spray of female TSM. Immediately following and two days after treatment no eggs hatched at either the field rate or one tenth field rate. However, by the fourth day egg hatch at the one tenth field rate treatment had risen to 56% and by the sixth day and thereafter eggs hatched at about the same rate as the control. Egg hatch from the field rate treatment took a longer time to recover. Only by the twentieth day after treatment did percentage hatch reach the control hatch level.

4.3.2.2 The Effect of Length of Exposure of Adult Female TSM to residues

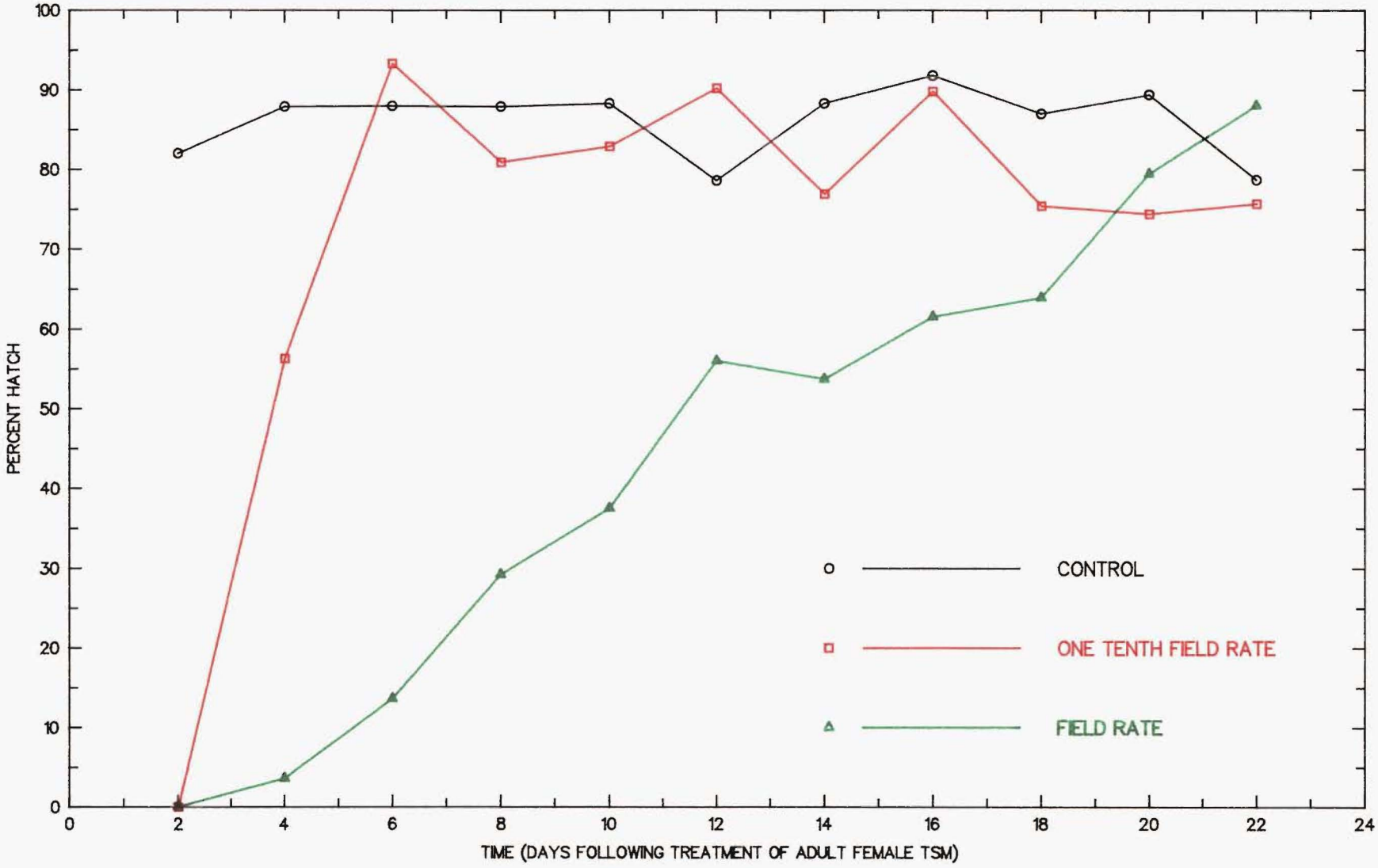
The effect on egg laying and hatch of female TSM exposed to residues of hexythiazox for varying lengths of time are summarised in Table 4.3.

Table 4.3: The effect of different periods of exposure to hexythiazox residues on egg laying and hatch of twospotted mite.

Exposure Time	Mean eggs laid/ female/day	SD	Mean eggs hatched/day	SD	Percentage hatch
1 days	5.87	1.1	4.61	1.36	78.5
3 days	5.25	1.93	4.03	1.96	76.8
5 days	4.62	1.84	3.86	1.81	83.5
Untreated Control	5.66	1.88	5.12	1.85	90.5
LSD 0.05	1.05		1.08		

The LSD test showed no significant difference in oviposition rate in any of the treatments compared with the untreated control. However,

FIGURE 4.1. PERCENTAGE HATCH OF EGGS PRODUCED BY ADULT FEMALE TSM AFTER DIRECT SPRAYING WITH HEXYTHIAZOX AT THE FIELD RATE (2.5 X 10^{-3} % a.i.) AND ONE TENTH FIELD RATE (2.5 X 10^{-4} % a.i.)



hatch rate was significantly ($p < 0.05$) lowered when female TSM were exposed to hexythiazox residues for three and five days, but not for one day, compared with the untreated controls.

Figure 4.2 shows the change in percentage hatch following removal of female TSM from spray residues. Eggs laid on spray residues and those laid two days after removal failed to hatch in any of the three chemical treatments. By the fourth day after removal percentage hatch rose to between 60 and 67% for all treatments, and by the sixth day to between 87 and 94% hatch. Percentage hatch for the three treatments remained high, about equal to control hatch, for the remainder of the experiment. There was no obvious difference in response between the three treatments.

4.3.3 Ovicidal Toxicity Tests

4.3.3.1 Comparison of Direct Spray with Residue Toxicity.

Table 4.4 shows the comparison of toxicity of hexythiazox between TSM eggs sprayed directly and eggs laid onto a spray residue.

Table 4.4: Comparison of toxicity of hexythiazox between TSM eggs direct sprayed and eggs exposed to a residue.

Treatment	LC_{50}*	95% C.L.	Slope	SE
Direct spray	1.6	1.0 - 2.3	2.18	0.08
Residue	3.2	2.5 - 3.9	3.83	0.13

* LC_{50} expressed in % a.i. x 10^{-4}.

Comparison of LC_{50} values showed hexythiazox to be twice as toxic to TSM eggs directly sprayed as to eggs laid on a spray residue. Figure 4.3 shows a comparison of log concentration-probit lines. The steeper slope of the residue regression line indicates a greater homogeneity of response compared with direct spray toxicity.

FIGURE 4.2. PERCENTAGE HATCH OF EGGS PRODUCED BY ADULT FEMALE TSM EXPOSED TO HEXYTHIAZOX RESIDUES (2.5×10^{-3} % a.i.) FOR VARYING LENGTHS OF TIME

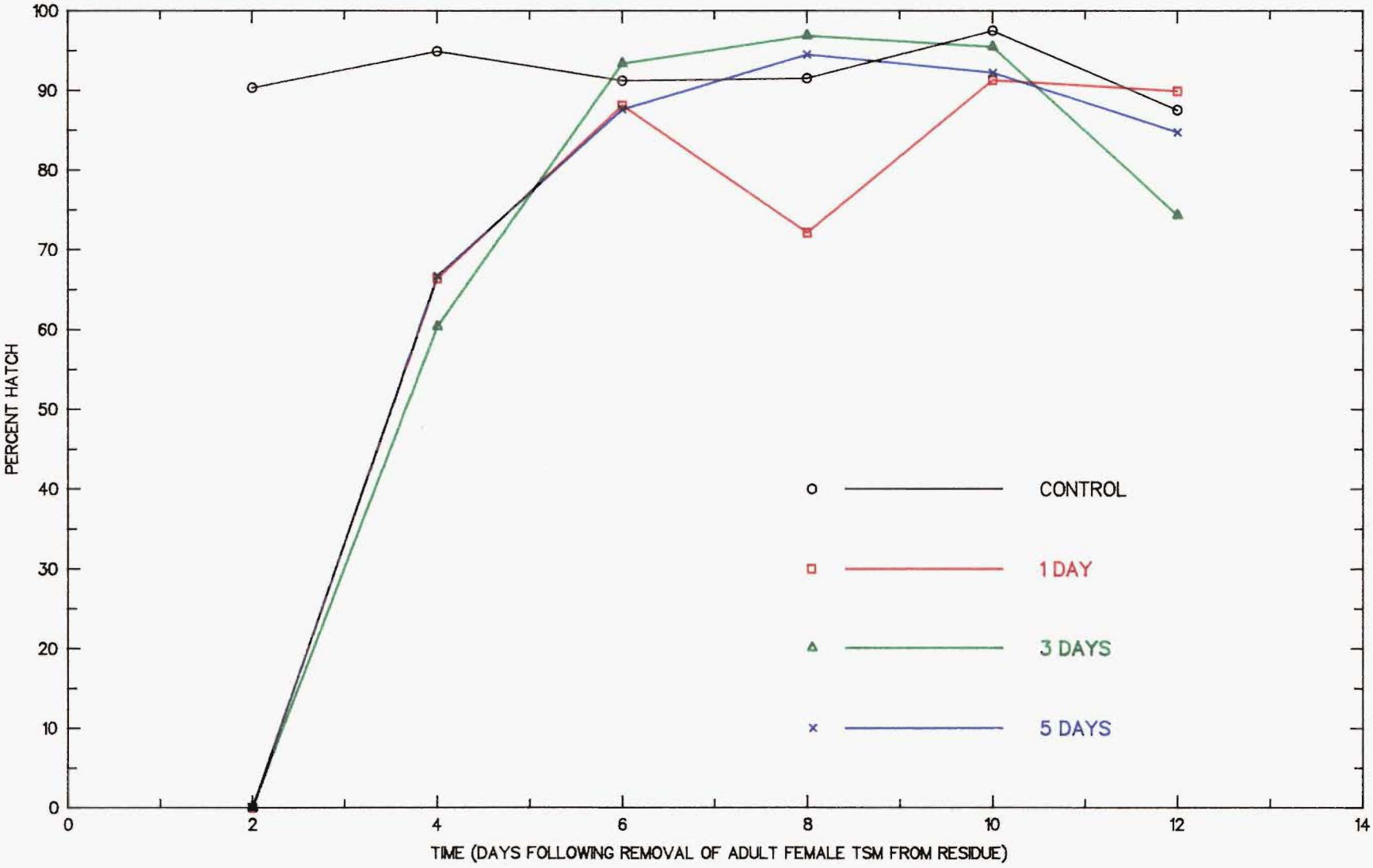
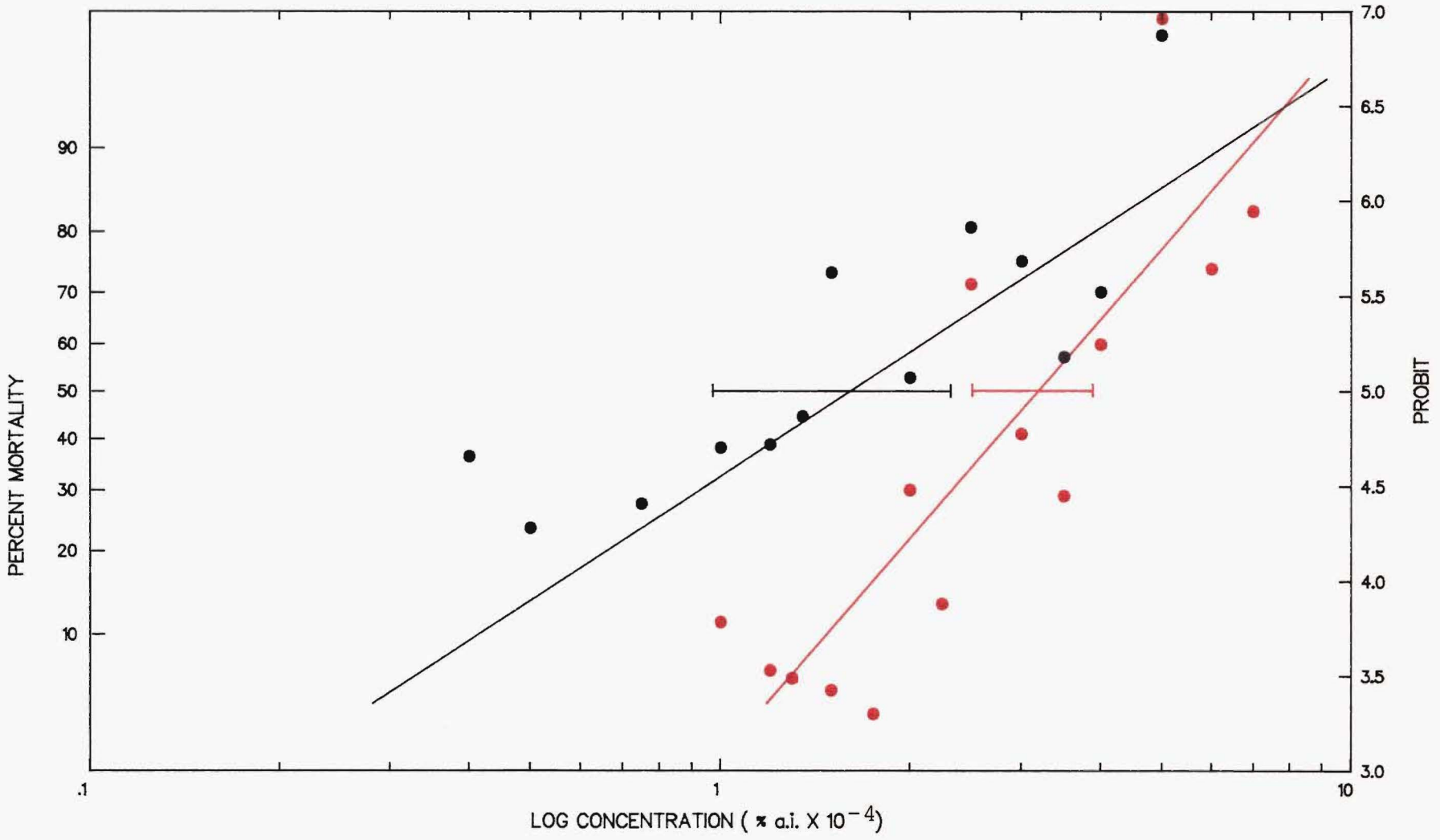


FIGURE 4.3. THE RESPONSES OF TSM EGGS TO DIRECT SPRAY AND EXPOSURE TO RESIDUE OF HEXYTHIAZOX



4.3.3.2 The Effect of Post-treatment Temperature on Toxicity

Results from probit analysis of the effect of post-treatment temperature on toxicity of hexythiazox are shown in Table 4.5.

Table 4.5: Effect of post-treatment temperature on toxicity of hexythiazox to twospotted mite eggs.

Post-treatment Temperature (°C)	LC_{50}*	95% C.L.	Slope	SE
15	0.3	0.2 - 0.5	2.41	0.13
20	1.0	0.7 - 1.3	2.27	0.08
25	1.6	1.0 - 2.3	2.18	0.08
30	2.6	1.9 - 3.6	2.05	0.06

* LC_{50} expressed as % a.i. x 10^{-4}.

Comparison of LC_{50} values shows an inverse relationship between temperature and toxicity, i.e., at higher temperatures TSM eggs became more tolerant. Eggs maintained at 30°C were 8.7 times more tolerant than eggs at 15°C. Regression analysis of the LC_{50} against temperature yielded the following equation (r^2 = 98.5%):

$$C = -2.01 + 0.151T$$

where: T = Temperature (°C)
C = LC_{50} Concentration (% a.i. x 10^{-4}).

Figure 4.4 shows a comparison of log-concentration probit lines for each post-treatment temperature.

4.3.3.3 The Effect of Egg Age on Toxicity

Results from probit analysis of the effect of egg age at the time of treatment on toxicity of hexythiazox are summarised in Table 4.6.

FIGURE 4.4. THE RESPONSES OF TSM EGGS DIRECTLY SPRAYED WITH HEXYTHIAZOX TO VARYING POST-TREATMENT TEMPERATURES

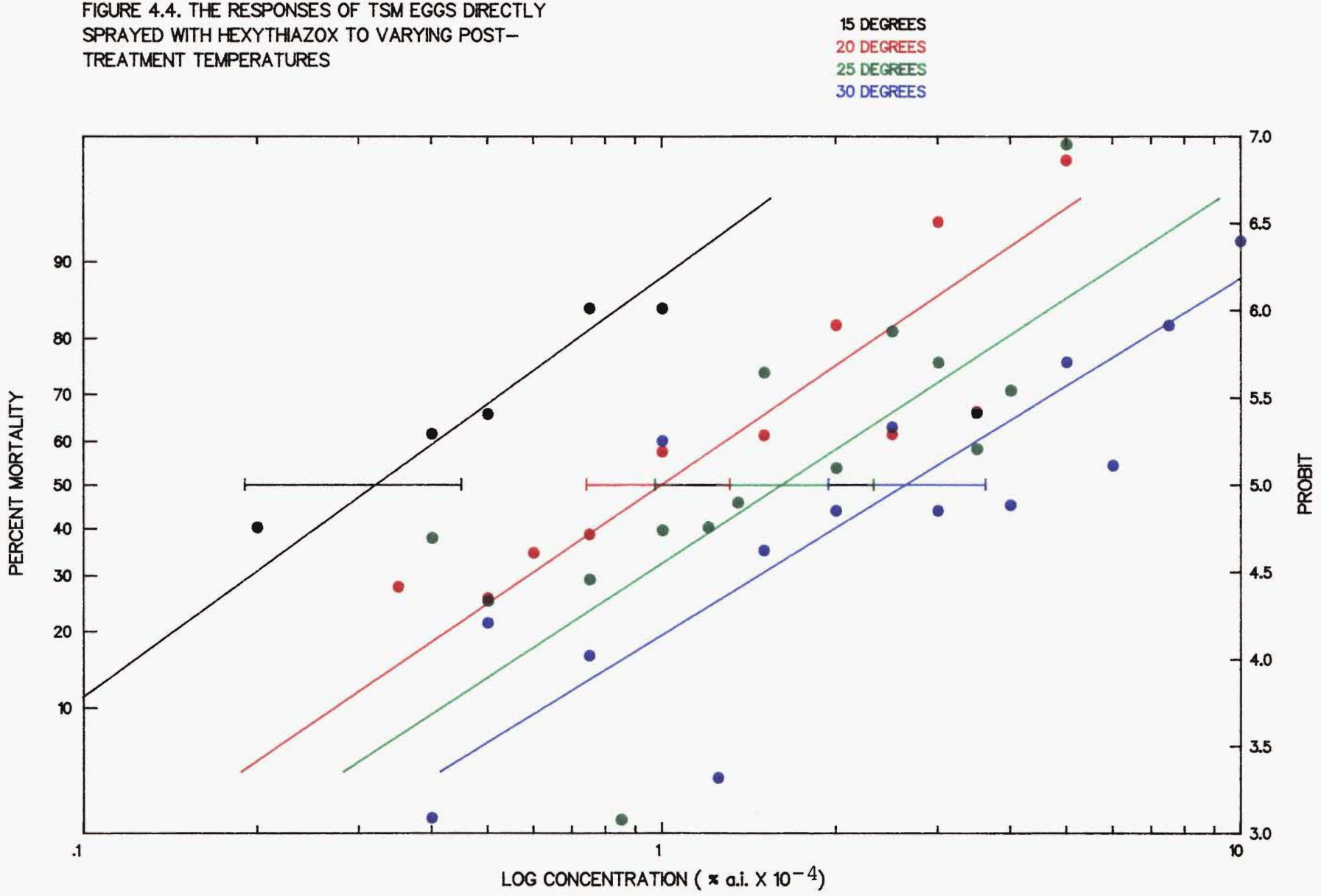


Table 4.6: The effect of egg age on the toxicity of hexythiazox.

Egg Age (hrs)	LC_{50}*	95% C.L.	Slope	SE
0-6	1.3	1.0 - 1.9	2.39	0.18
6-12	1.7	1.4 - 2.2	2.99	0.17
12-24	1.6	1.0 - 2.6	1.72	0.08
24-48	2.6	1.6 - 3.7	2.00	0.07
48-72	5.2	3.4 - 7.1	1.85	0.07
72-96	634.2	392.6 - 1177.4	0.77	0.04

* LC_{50} expressed as % a.i. x 10^{-4}.

Although there was no significant difference ($p > 0.05$) in toxicity between the first four egg age groups; 0-6, 6-12, 12-24 and 24-48 hours, there was a general increase in tolerance to hexythiazox with age. There was a four-fold increase in tolerance from eggs treated at 0-6 hours compared with those treated at 48-72 hours. Between this group and the next (72-96 hours), the increase in tolerance was more than 100-fold.

Regression of the mid points of the first five age groups against $\log_e$ of the LC_{50} values yielded the following equation ($r^2 = 97.3\%$):

$$C = 0.202 + 0.0232A$$

where: A = midpoint of the egg age period (hrs)
C = Log_e LC_{50} concentration (% a.i. x 10^{-4}).

Figure 4.5 shows the log-concentration probit lines for all egg age groups except 72-96 hours. The 72-96 hour egg age group is included in Figure 4.6. (Note: some data points in this graph have been omitted for clarity).

4.3.3.4 The Effect of Leaf Type on Toxicity

Results from probit analysis of the effect of leaf type on toxicity of hexythiazox to TSM eggs are shown in Table 4.6.

FIGURE 4.5. THE RESPONSES OF TSM EGGS OF VARYING AGES TO DIRECT SPRAYS OF HEXYTHIAZOX

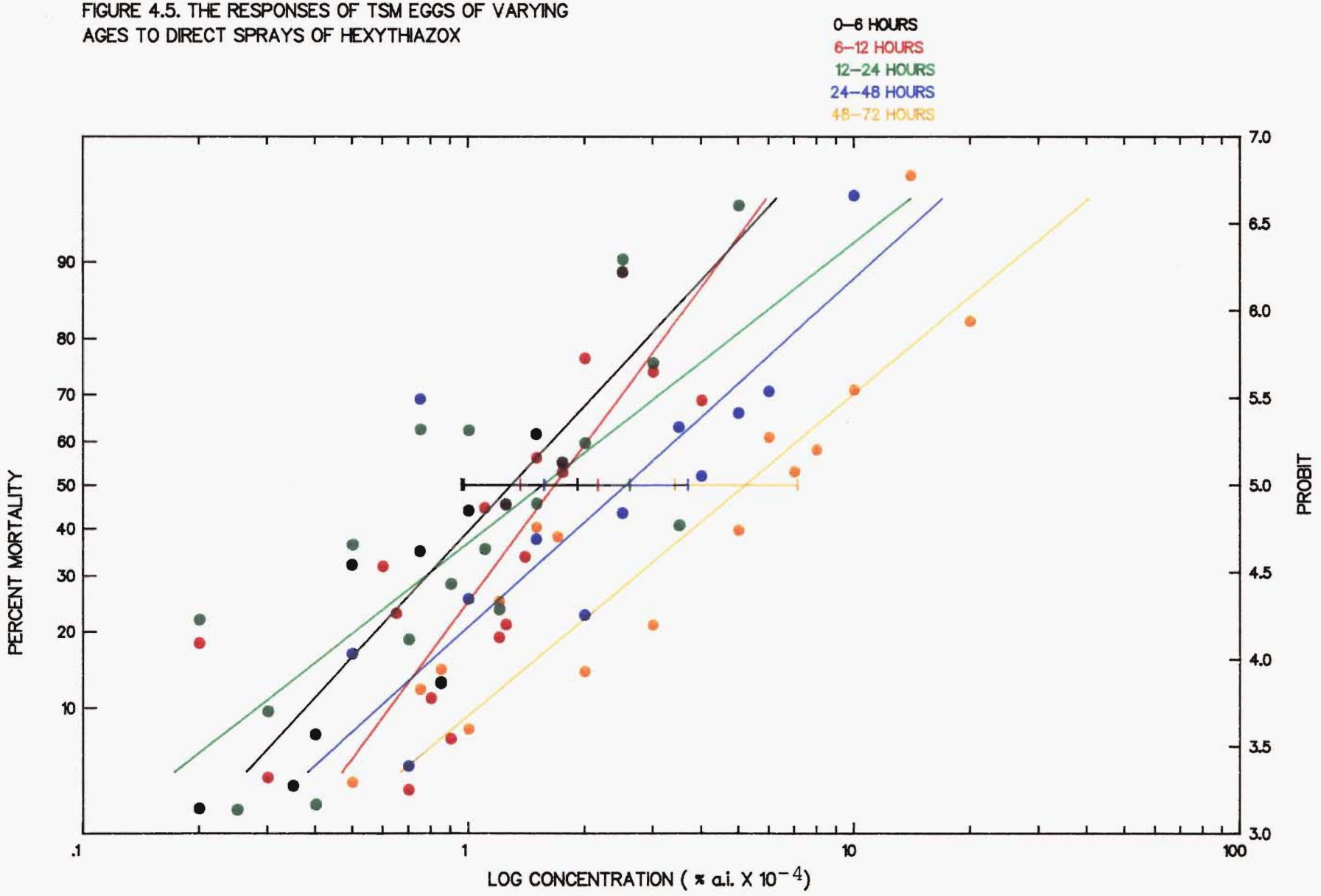


FIGURE 4.6. THE RESPONSES OF TSM EGGS OF VARYING AGES TO DIRECT SPRAYS OF HEXYTHIAZOX

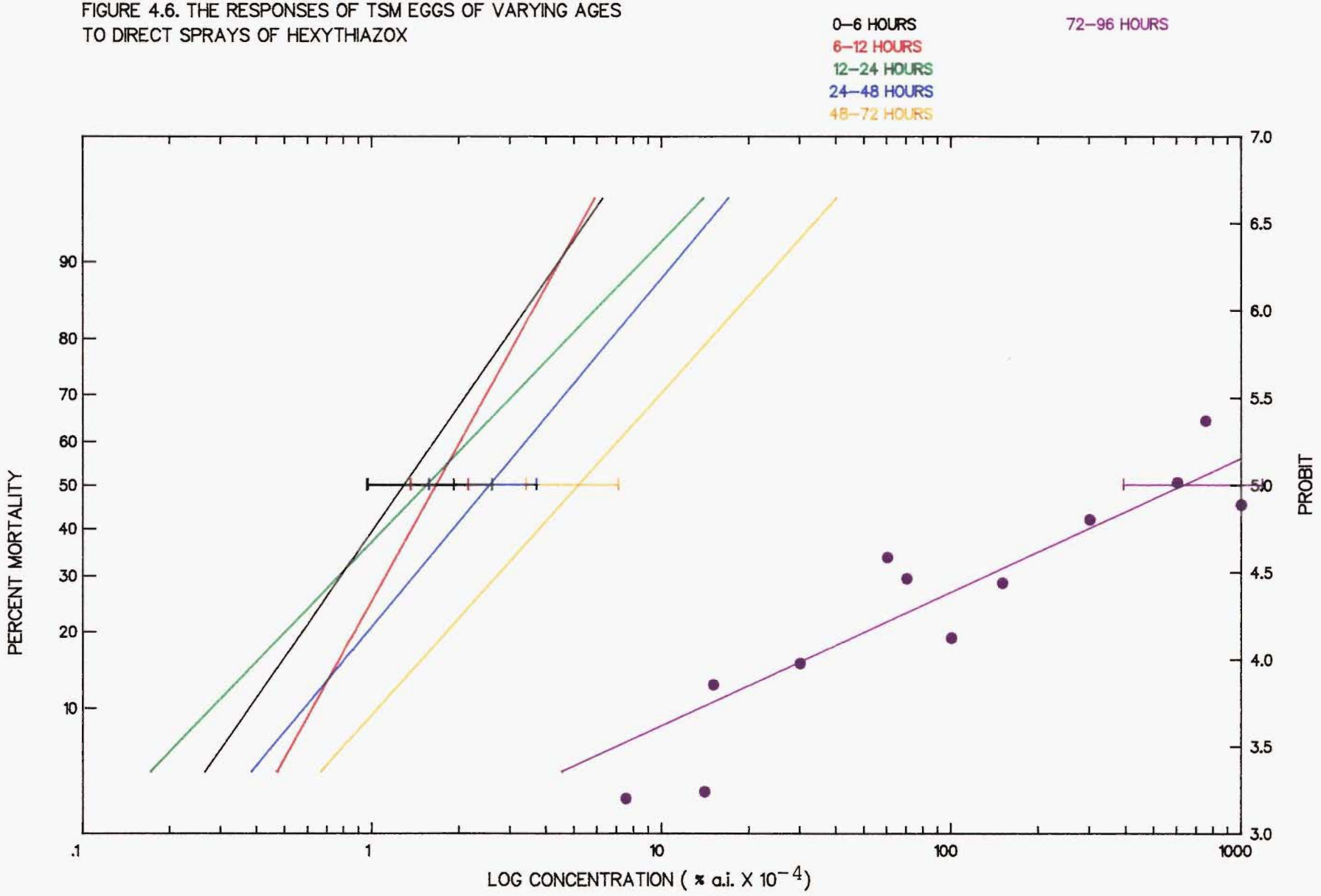


Table 4.6: The effect of leaf type on toxicity of hexythiazox to TSM eggs.

Leaf type	LC_{50}*	95% C.L.	Slope	SE
Broad Bean	0.34	0.30 - 0.40	2.79	0.18
Raspberry	1.14	0.95 - 1.31	3.38	0.14
Strawberry	1.03	0.93 - 1.15	3.00	0.16
Apple - 'Red Delicious'	1.87	1.54 - 2.26	2.64	0.11
Apple - 'Granny Smith'	2.12	1.61 - 2.86	2.37	0.17

* LC_{50} expressed as % a.i. x 10^{-4}.

Comparison of LC_{50} values shows that eggs laid on broad bean leaves were most susceptible. There was no significant difference ($p > 0.05$) in LC_{50} between eggs laid on raspberry and strawberry leaves; both were about three times more tolerant than eggs on broad bean leaves. There was no significant difference ($p > 0.05$) in toxicity of eggs between the two apple cultivars. Eggs on the apple cultivars were 1.8 times more tolerant than eggs on raspberry or strawberry leaves and 5.9 times more tolerant than eggs on broad bean leaves.

Figure 4.7 shows log-concentration probit lines for each leaf type.

Plates 3-7 show scanning electron micrographs of TSM eggs on each of the five leaf types. Broad bean leaves (Plate 3) were the smoothest of those tested and were notable for their lack of leaf hairs. Although similar to broad bean in overall lamina structure, the strawberry leaves (Plate 4) had long straight hairs running along the leaf veins. The two apple cultivars (Plates 5 and 6) were very similar in lamina structure, both being densely covered in hairs which gave the underside of the leaves a furry appearance. While no quantitative assessment was made, the 'Granny Smith' leaves appeared slightly more hairy than the 'Red Delicious' leaves. The lamina of the apple cultivars appeared similar

FIGURE 4.7. THE RESPONSES OF TSM EGGS LAID ON DIFFERENT LEAF TYPES TO DIRECT SPRAYS OF HEXYTHIAZOX

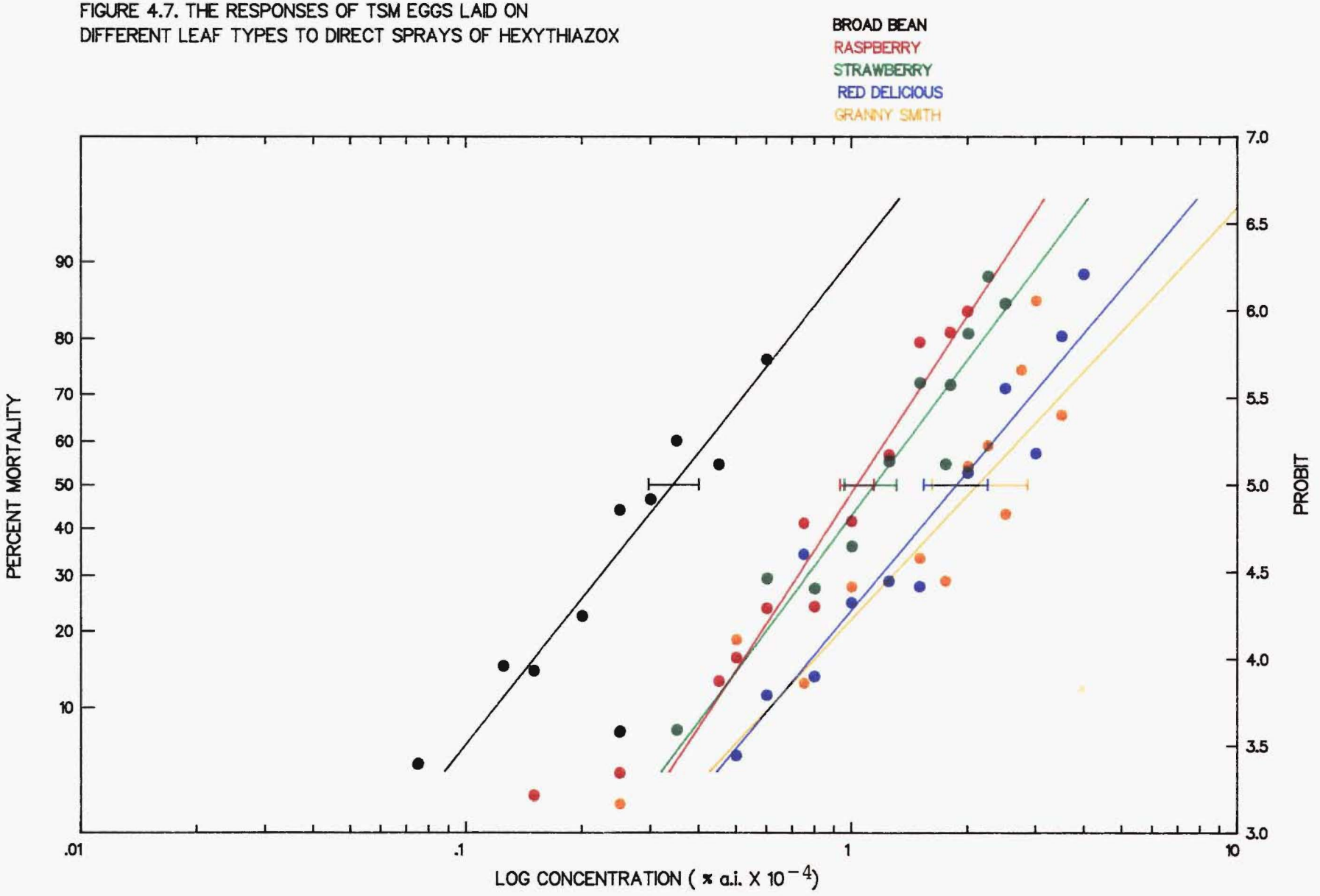
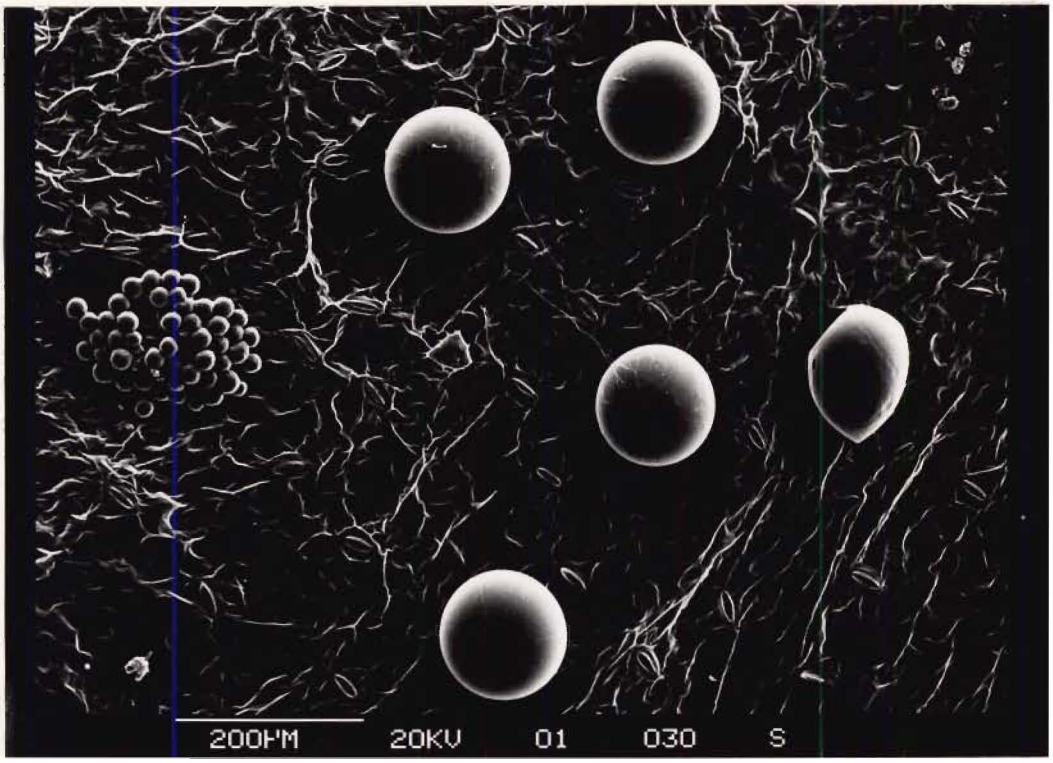


PLATE 3: Scanning electron micrograph of TSM eggs laid on a broad bean leaf disc

PLATE 4: Scanning electron micrograph of a TSM egg laid on a strawberry leaf disc


200µM 20KV 01 030 S

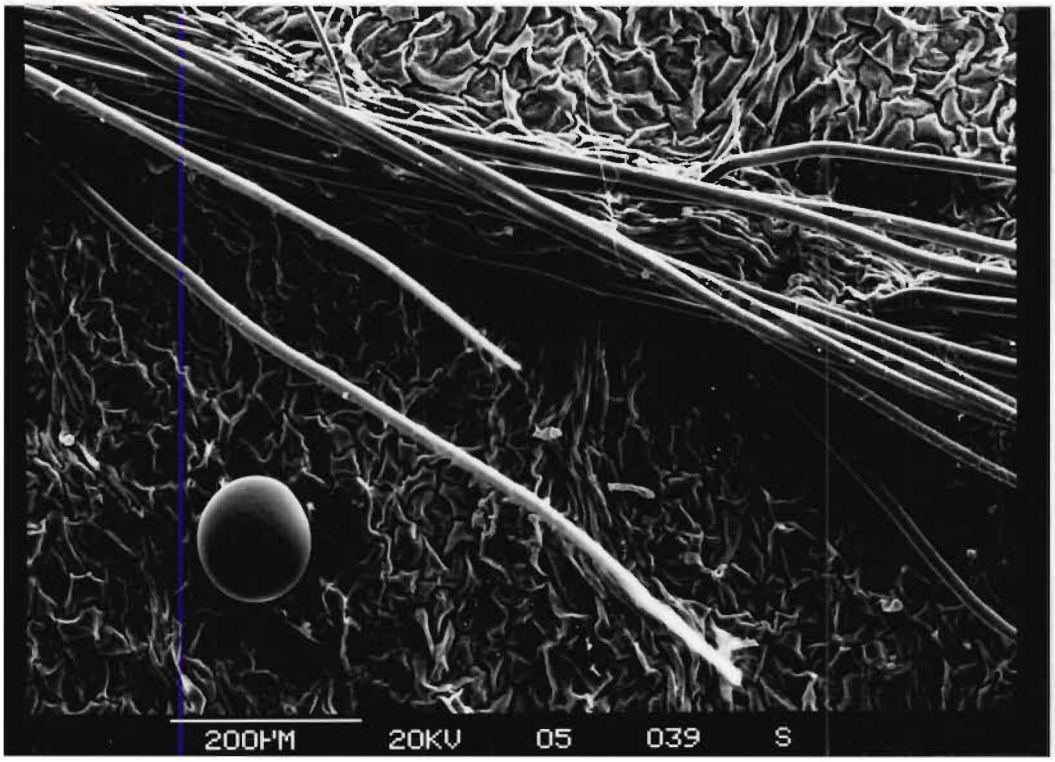
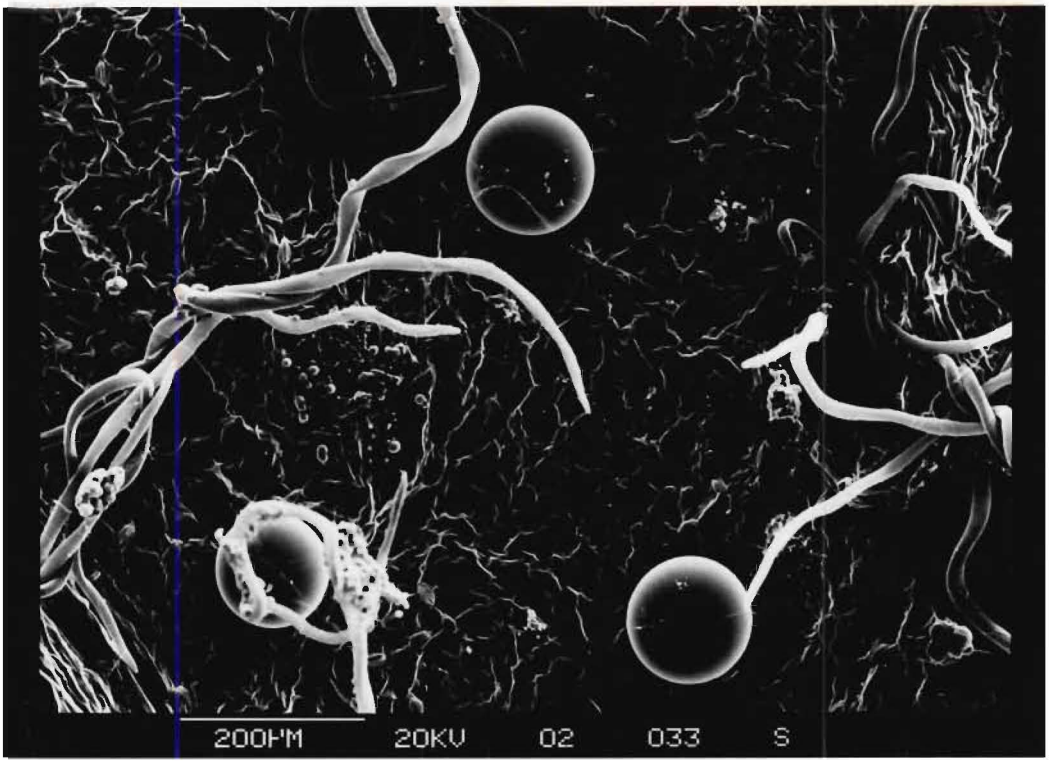

200µM 20KV 05 039 S

PLATE 5: Scanning electron micrograph of TSM eggs laid on a 'Red Delicious' leaf disc

PLATE 6: Scanning electron micrograph of TSM eggs laid on a 'Granny Smith' apple leaf disc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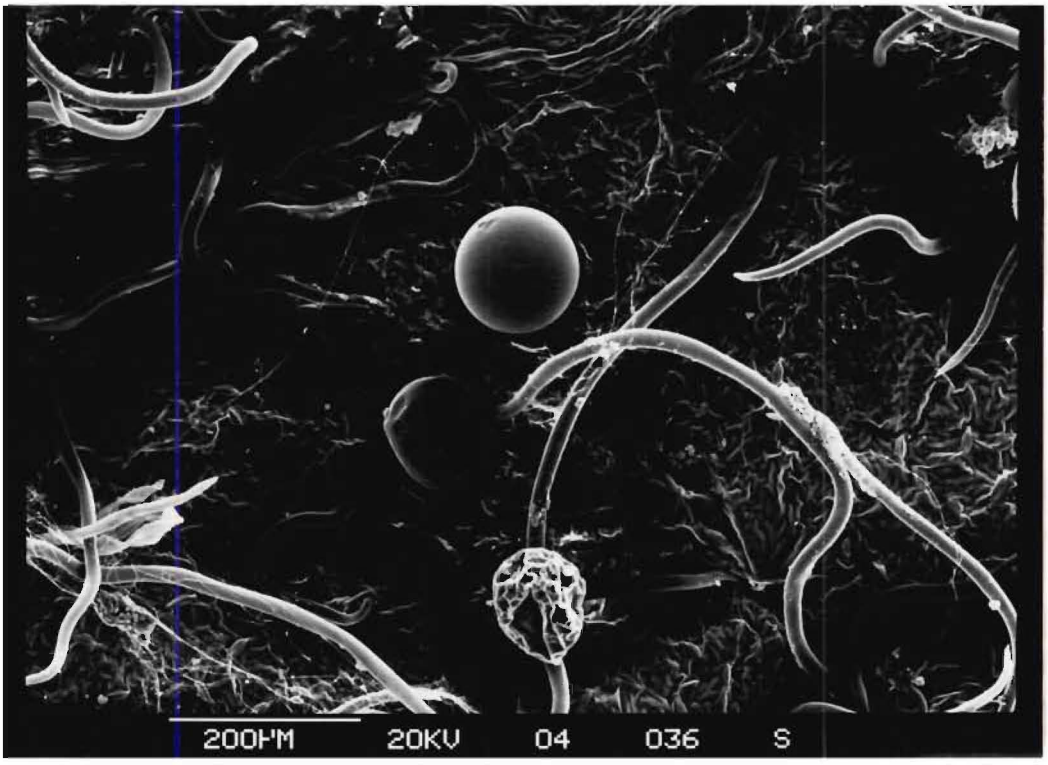

200µM 20KV 04 036 S

to those of the broad bean and strawberry although somewhat more convoluted. The entire underside of the raspberry leaves (Plate 7) were covered in a dense mat of hairs, so that the lamina was not visible.

Almost without exception, TSM eggs were laid on the lamina between the leaf hairs for all leaf types except raspberry, where the leaf surface was not accessible to the mites.

4.4 DEVELOPMENT OF TREATED TSM EGGS

4.4.1 Light Microscopy

Plate 8 shows a killed embryo within a treated TSM egg. All the major visible body structures such as the body segments, legs, pedipalps, rostrum, chelicera and setae were visible. A dark area located centrally in the hysterosoma was another prominent feature found in both treated and untreated eggs. This was thought to be an area of uric acid crystal storage.

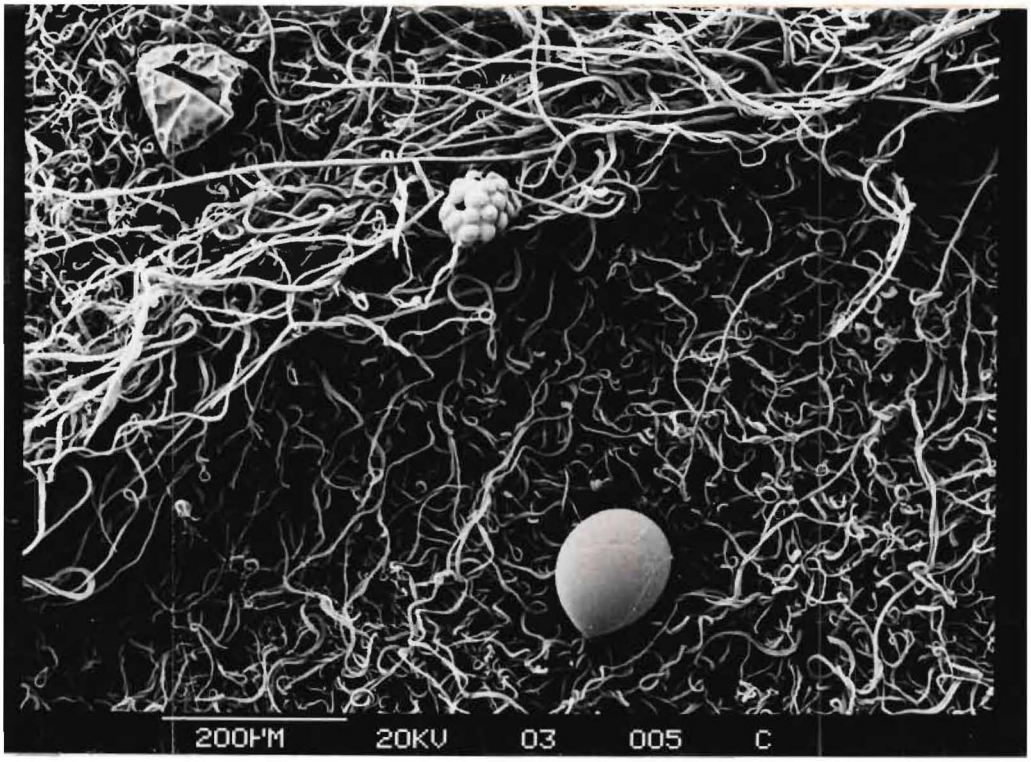
Plate 9 shows an embryo removed from its egg shell, showing the closeness in development of the embryo to a newly-emerged larva.

4.4.2 Electron Microscopy

Investigation of both treated and untreated TSM eggs showed no apparent differences in surface structure. Apart from webbing laid down by adult mites the only interruption in the smooth egg surface was a protruberance of the egg shell due to penetration by the perforation organs described by Dittrich (1969, 1971) (Plate 10).

PLATE 7: Scanning electron micrograph of a TSM egg laid on a raspberry leaf disc

PLATE 8: Micrograph of a TSM egg killed by hexythiazox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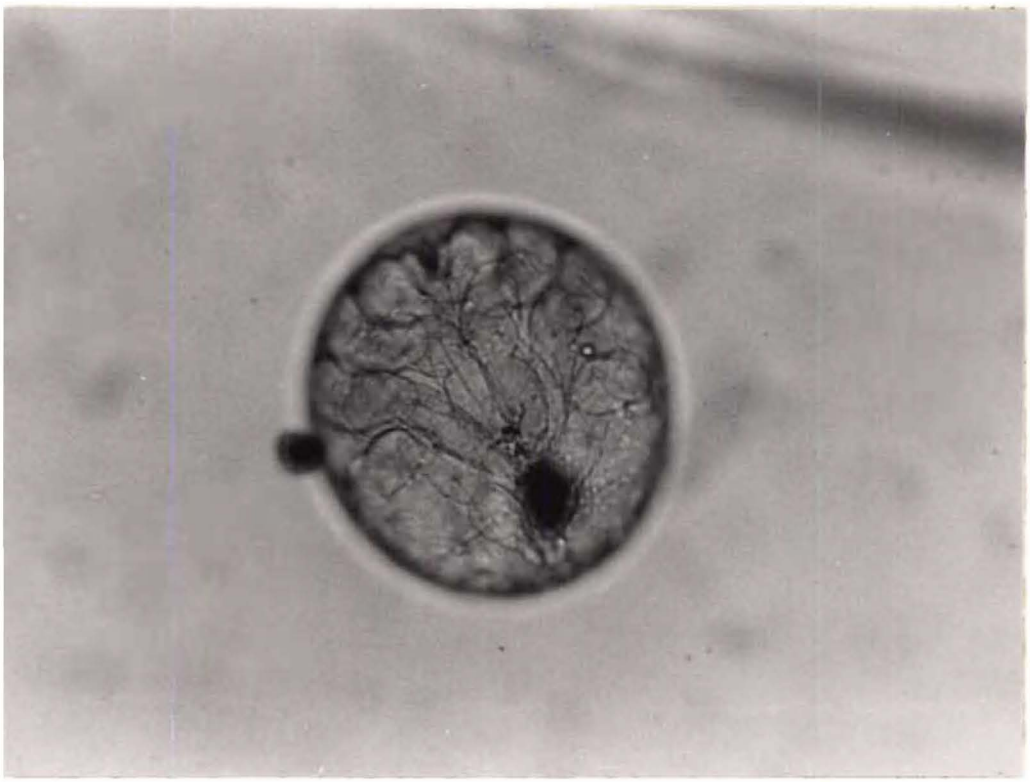
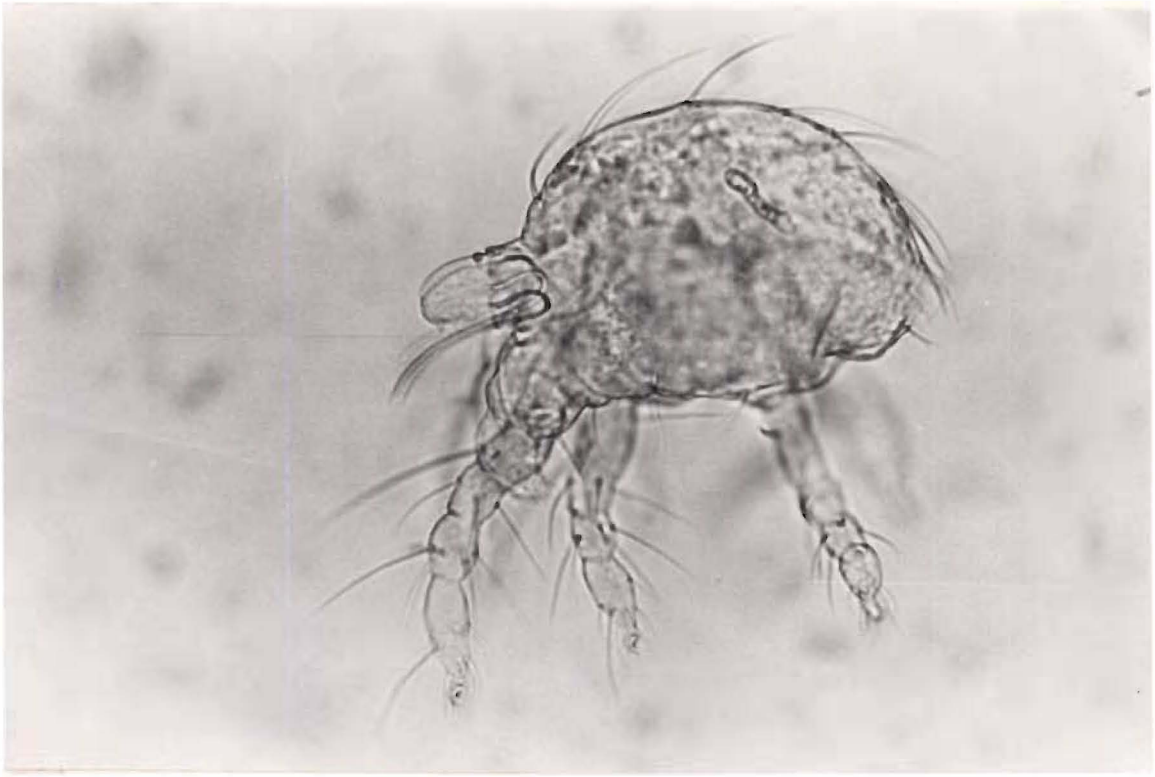
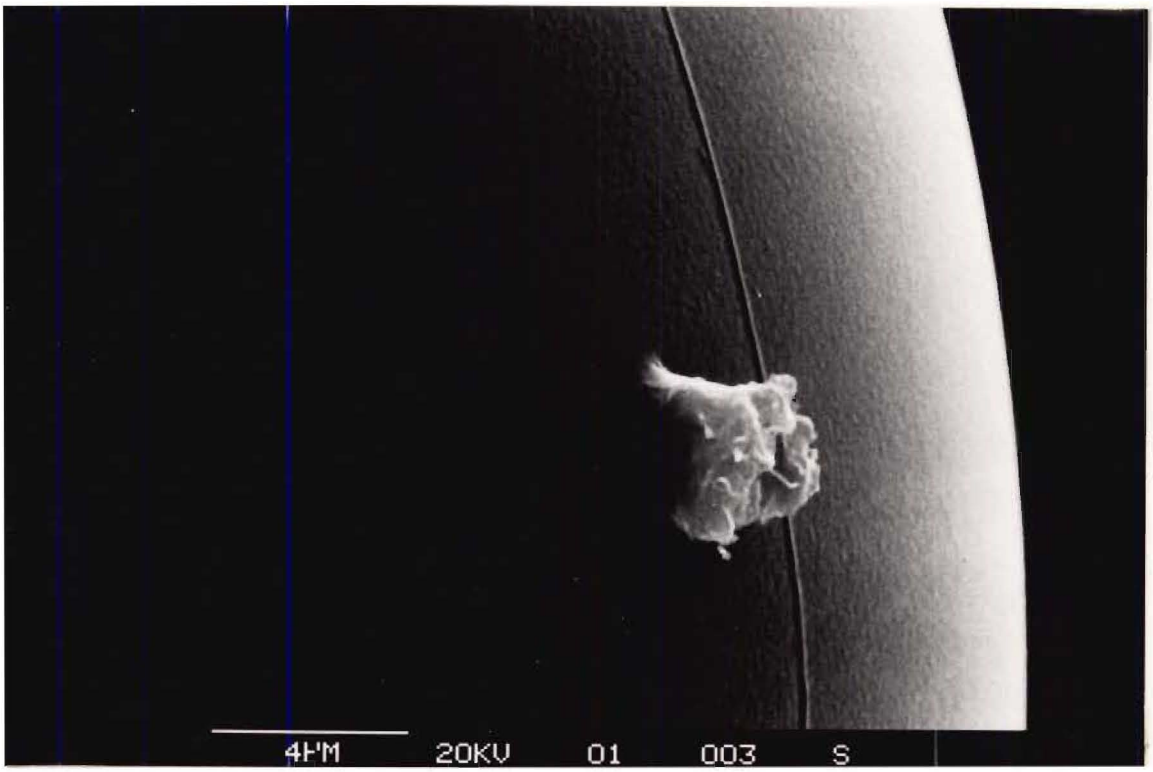


PLATE 9: Micrograph of a TSM embryo killed by hexythiazox and removed from its egg shell

PLATE 10: Scanning electron micrograph of a TSM egg killed by hexythiazox showing the perforation organ

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CHAPTER 5
DISCUSSION

5.1 THE TOXICITY OF HEXYTHIAZOX TO ADULT FEMALE TSM

Hexythiazox may be considered essentially non-toxic to adult female TSM. Since the calculated LC_{50} value was 59 times greater than the suggested field rate (0.025 g a.i. L^{-1}) it is unlikely that any significant mortality to adults would occur at field rate levels. Other ovicides such as chlorfenson, tetradifon, cycloprate and clofentezine have also been shown to be relatively non-toxic to adult mites (Ebeling and Pence, 1954; Overmeer, 1967; Asano and Kamei, 1979; Aveyard *et al.*, 1986).

5.2 THE STERILISING EFFECT OF HEXYTHIAZOX ON ADULT FEMALE TSM

Hexythiazox was shown to function as a chemosterilant. Adult female TSM produced fewer viable eggs for the duration of the experiment following treatment. However, this effect was found to be temporary. Percentage hatch of eggs produced by adult female TSM following treatment and removal from the residue rose to a level equivalent to that of the controls. The speed of recovery was dependent on the rate of hexythiazox applied. Mites sprayed at field rate took about 20 days to recover to control levels, whereas adults treated at one-tenth field rate recovered after six days. Similar responses have been found for TSM treated with tetradifon (Batth and Davidson, 1959) and clofentezine (Chapman and Marris, 1986). Direct spraying of adult female TSM also caused a 30% reduction in the oviposition rate at both concentrations used.

Exposure of adult female TSM to hexythiazox residues did not significantly influence egg viability, although the numbers of eggs laid by females exposed to residues for five days were significantly lower than for those on untreated leaf discs. Recovery of egg viability was not found to be dependent on the length of exposure of females to hexythiazox residues. The oviposition rate of the mites on treated surfaces was not significantly different from the controls at all exposure periods.

5.3 OVICIDE BIOASSAY METHODOLOGY

The method used in this study for the bioassay of hexythiazox against TSM eggs was a leaf disc method adapted from that described by Overmeer and van Zon (1973). Harrison and Smith (1961) criticised the use of leaf or leaf disc methods because of the complicating effects caused by transpiration and radiant heat which, they argued, introduces unnecessary variability. Indeed, Harrison and Smith (1961) showed that humidity was a significant factor in the toxicity of the ovicides tested. However, there are limitations to the use of an inert substrate such as a glass microscope slide as suggested by Harrison and Smith (1961). Transferral and retention of adult mites for a period of oviposition in a glass slide cage is particularly difficult and time-consuming and mites are unable to feed as they can on a leaf substrate. While feeding is not important during the oviposition period, it is necessary if mortality is to be examined beyond emergence from the egg.

A further factor in favour of the use of a leaf substrate is that it more closely reflects the field situation. Fisher and Wrensch (1986) noted that use of a leaf substrate approximates a natural route of exposure; any interactions occurring between the leaf, leaf cuticle, and the toxin that may alter the toxicity of the acaricide are accounted for in this method. This is further borne out by studies which have shown the importance of the interaction between spray deposits and leaf surfaces on the action of pesticides (Munthali, 1982; Munthali and Scopes, 1984; Stevens and Baker, 1987).

For the above reasons the criticisms of Harrison and Smith (1961) were rejected and the leaf disc bioassay method adopted.

Leaf discs were used in preference to whole leaves for several reasons. Use of leaf discs simplified the location and counting of eggs which may be difficult if a large surface is used. Greater uniformity of leaf surface could also be ensured. For example, use of the leaf midribs was avoided as this has been found to cause uneven distribution of residues when leaf dipping methods are used (Dittrich, 1962). The small size of leaf discs also facilitates the use of large numbers of discs which improves the statistical validity of results (Fisher and Wrensch, 1986).

Use of a spray tower, such as the Potter tower used in this study, is generally considered to be preferable to dip methods (Helle and Overmeer, 1985). Spray tower applications more closely approximate field conditions (Suckling, 1983) and allow manipulation of variables such as droplet size and density. Experiments by Dittrich (1962) showed that a cage spray method (similar to the leaf-disc spray method used in this study) was less variable than either a slide dip or leaf dip method.

Experiments performed in the early part of this study tended to be more variable than those performed later. Part of this variability can be attributed to the performance of the Potter tower, because calibration experiments showed that spray deposits differed significantly throughout this study. This was overcome by the adjustments made to the Potter tower described in 3.4.2.

Difficulties encountered in the use of the Potter tower in this study were probably attributable to the age of the machine used rather than any inherent shortcomings of the design. In retrospect, further information regarding spray deposits (droplet size and density) would have been of some use, particularly in light of studies by Munthali (1984) and Munthali and Scopes (1982) which have emphasised the important effect of spray deposits on toxicity.

5.4 OVICIDAL TOXICITY TESTS

5.4.1 The Effect of Direct Spray and Residue Exposure of Hexythiazox on TSM Eggs

Baseline toxicity data were established for TSM eggs treated by direct spray and residual exposure to hexythiazox. The LC_{50} value for direct spray exposure was 1.6×10^{-4}% a.i. By comparison Anon. (1984) calculated an LC_{50} value for TSM of 3.4×10^{-5}% a.i. using what was described as a detached leaf method (no further information was given). Welty *et al.* (1987) calculated an LC_{50} value of 2.2×10^{-4}% a.i. for summer eggs of the European red mite (*Panonychus ulmi*) using a leaf disc method similar to that used in this study, although eggs were treated by dipping leaf discs into hexythiazox suspensions. An LC_{50} value of 2.0×10^{-3}% a.i. was calculated for *P. ulmi* winter eggs.

In the leaf type experiment an LC_{50} value for TSM eggs was again determined. This was done following modifications to the Potter tower. The calculated LC_{50} value was 3.4×10^{-5}% a.i., the same as determined by Anon. (1984) and 4.7 times less than initially estimated. The differences in LC_{50} values can be attributed to the improved spray coverage following modifications. The variation observed highlights the need for standardisation of conditions for valid comparisons to be made.

In comparison with other ovicides and ovo-larvicides, hexythiazox is of high toxicity to TSM eggs. For example, the bridged diphenyl compounds, chlorobenzilate (LC_{50} 7.8×10^{-2} to 1.26×10^{-1}% a.i.; Ebeling and Pence, 1954) and dicofol (LC_{50} 1.2×10^{-3}% a.i.; Abul-Hab and Stafford, 1961), were found to be from 7.5 to more than 750 times less toxic to TSM eggs than hexythiazox. The sulphur derivatives, tetrasul (LC_{50} 1.0×10^{-5}% a.i.; Overmeer, 1967), tetradifon (1.1 to 2.9×10^{-4}% a.i.; Overmeer, 1967; Harrison and Smith, 1961) and chlorfenson (LC_{50} 5.7×10^{-4}% a.i.; Harrison and Smith, 1961) were found to be of similar toxicity to TSM eggs as hexythiazox. Cycloprate (LC_{50} 4.0×10^{-3}% a.i.; Staal *et al.*, 1975) was found to be 25 times less toxic than that of hexythiazox, while clofentezine was found to be 10 times more toxic than hexythiazox (LC_{50} 1.6×10^{-5}% a.i.; Aveyard *et al.*, 1986).

It should be noted that the method used to determine the toxicity of hexythiazox measured only direct ovicidal activity. This may result in a conservative estimate of toxicity, as mortality may occur following hatch. No attempt was made to determine larval mortality which appeared to be insignificant. Recent evidence (Chapman, unpublished report) has revealed that hexythiazox kills TSM at the nymphochrysalis stage.

Mortality of eggs due to direct spray exposure to hexythiazox was found to be twice that due to residue exposure (LC_{50} 3.2×10^{-4}% a.i.). Similarly, Asano and Kamei (1977) found that mortality of *Panonychus citri* eggs was greater from direct spray exposure to cycloprate than from residue exposure. For example, at a concentration of 6.25×10^{-3}% a.i., direct spray exposure resulted in 62.9% hatch while 85.2% hatch occurred following residue exposure. Conversely, Meltzer and Dietvorst (1957) found *Panonychus ulmi* eggs to be more susceptible to residue exposure than those dipped in tetradifon solutions. However, it was suggested that the difference in response may be due to differences in the ages of eggs

between the two treatments which may affect susceptibility. Harrison and Smith (1961) found the susceptibility of TSM eggs treated with tetradifon to decrease with age. A further reason suggested was that an increase in toxicity may have occurred due to the chemosterilant action of tetradifon. However, if this was true similar results might have been expected for hexythiazox which has also been found to have chemosterilant properties.

It is unclear why higher egg mortality resulted from direct spray than residue exposure. Although direct spray would be expected to cause higher mortality due to direct hits of spray droplets on eggs, Munthali and Scopes (1982) showed that, while eggs directly hit invariably died, the proportion of eggs directly hit was less than 10% even at a droplet density of 200 cm^{-2}. No information is available on the droplet densities produced in this study.

5.4.2 The Effect of Post-Treatment Temperature on Toxicity

An inverse relationship between post-treatment temperature and toxicity of hexythiazox to TSM eggs was found over the temperature range tested of 15 to 30°C. A reason for this response may be that mortality is dependent on the penetration of hexythiazox into the egg. That being the case, and the fact that eggs take longer to develop at low temperatures (mean hatch time 389 hours at 15°C, 78 hours at 30°C), the time available for the penetration of hexythiazox into the egg would increase with a decrease in temperature. The toxicity of several other pesticides, such as the synthetic pyrethroids, has similarly been found to be inversely related to temperature (Sparks *et al.*, 1982).

5.4.3 The Effect of Egg Age on Toxicity

An inverse relationship between egg age at the time of treatment and toxicity of hexythiazox was found. Although the decrease in toxicity of hexythiazox to eggs over the 0 to 72 hour age range was relatively small (a four-fold decrease), eggs in the 72-96 hour age range decreased in susceptibility by a factor of more than 100 over the previous age group (48-72 hours), and by a factor of more than 450 times that of the first age group (0-6 hours). Similar egg age-toxicity responses have also been found in hexythiazox treated eggs of *Tetranychus*

pacificus (Hoy and Ouyang, 1986) and *Panonychus ulmi* (Welty *et al.*, 1987).

The reason for the marked decrease in susceptibility of 72-96 hour old eggs was not investigated. As this age group was close to the hatch point, and some larvae had already emerged before treatment, it may be that hexythiazox had insufficient time to penetrate the egg and kill the embryo. The possible importance of the rate of hexythiazox penetration was discussed in the previous section with respect to the incubation temperature. However, at present little information is available on the penetration of ovicides into mite eggs. Hopp (1954) attributed differences in the toxicity of three ovicides (chlorobenzilate, p-chlorophenyl benzene sulphonate and chlorfenson) to differences in the rates of penetration between the chemicals.

Alternatively, changes in susceptibility with egg age may be linked with physiological changes occurring in the embryo or egg shell. To determine this, however, detailed information on the mode of action of hexythiazox is necessary.

5.4.4 The Effect of Leaf Type on Toxicity

Leaf type was found to be a significant factor affecting the toxicity of hexythiazox to TSM eggs. Ovicidal activity for the different leaf types decreased in the following order: broad bean, raspberry, strawberry, 'Red Delicious' apple, 'Granny Smith' apple. A maximum 6.2-fold difference in LC_{50} values occurred between broad bean and 'Granny Smith' apple leaves. Similarly, Asano and Kamei (1982) examined the effect of leaf type on the toxicity of cycloprate to TSM and *Panonychus ulmi* eggs using a range of leaf types. Leaf type was found to be a significant factor, accounting for up to a four-fold difference in toxicity.

Differences in toxicity could not be explained by differences in leaf hair density. Scanning electron micrographs showed broad bean leaves to be hairless; strawberry leaves were similar except for long straight hairs along the leaf veins. The two apple cultivars had relatively more hairs of a pilose nature covering the underside of the leaf while the raspberry leaves were covered in a dense matting of hairs. Thus if hairiness was correlated with toxicity it would be expected that LC_{50} values of broad bean and strawberry would be similar,

the LC_{50} of raspberry at the other extreme and the two apple cultivars to be intermediate. This was not the case. Asano and Kamei (1982) investigated the toxicity of cycloprate to TSM eggs on the upper and lower leaf surfaces of apple and peach leaves. No difference in toxicity was found to occur between the peach leaf surfaces, which were identical with respect to hairiness. However, the toxicity of cycloprate on apple leaves was found to be about three times greater on the upper surface, which was hairless, than the lower surface, which was relatively hairy. The differences in toxicity between the upper and lower surfaces of apple were thought to be due to differences in hairiness. However, differences in ovicidal activity on other leaf types could not be accounted for by the hairiness of the leaf surface.

Munthali and Scopes (1982) showed that, for a stationary target such as a mite eggs, the biological activity of a pesticide applied as an ultra-low volume spray must rely on the spread of pesticide on, or through, the leaf after impaction of the droplet (or by fumigant effect). Therefore the translaminar action of a pesticide has an important effect on toxicity. Although no specific studies on the mobility of hexythiazox on different leaf types have been carried out, insight may be gained by considering the mobility of other pesticides. For example, Stevens and Baker (1987) examined the foliar spread of three herbicides on apple, bean and strawberry leaves. The spread factor of the herbicides on bean leaves was greatest, followed by strawberry and then apple. This corresponds with the relative order of toxicity of hexythiazox to TSM eggs on these leaf types. Thus differences in the toxicity of hexythiazox may be related to the ability of the leaf to transport the spray residue to the target. It is unknown whether the variation in toxicity is attributable to the active ingredient or the surfactants in the formulation.

A further factor affecting the toxicity of an ovicide is applied spray volume. Wakou and Sugawara (1974) found the LC_{50} values for dicofol applied to TSM eggs varied with leaf type (apple, bean and peach) and spray volume applied. At low spray volume levels differences in toxicity between the three leaf types were apparent. Eggs on peach leaves were most susceptible, followed by bean and apple. At higher spray volumes no differences in toxicity were observed. Furthermore, the susceptibility of eggs on leaves dipped in dicofol solution was reversed. Eggs on apple leaves were most susceptible, followed by bean

and peach. Therefore, the effect of leaf type on toxicity is dependent on the application method (i.e. whether sprayed or dipped) and, if dipped, on the water volume applied.

5.5 THE EFFECT OF HEXYTHIAZOX ON THE DEVELOPMENT OF TSM EGGS

There was no apparent difference in development between eggs treated with a lethal dose of hexythiazox and control eggs. However, treated eggs failed to hatch. Embryos killed by hexythiazox were morphologically indistinguishable from newly-emerged larvae. The appearance of the perforation organs, revealed by SEM examination, in treated eggs further indicates the advanced stage of development reached before mortality occurs. Dittrich (1971) found that the perforation organs emerged about 68 hours after deposition (at 28°C), while hatching occurred at around 76 hours after deposition.

Chapman (1986) and Welty *et al.* (1987) noted that immature mite stages treated with hexythiazox died at the chrysalis stage. This suggests that hexythiazox acts on physiological processes common to late stages of embryogenesis and the moulting stages, such as chitin formation. However, detailed biochemical study is necessary to elucidate this.

5.6 PRACTICAL IMPLICATIONS OF LABORATORY RESULTS

Although the conditions of laboratory experiments may differ markedly from field conditions, inferences about the field performance of a chemical may be made from laboratory results. Haverty and Robertson (1982) developed a method by which field application rates could be determined from laboratory data. This involved estimation of the MED_{90} (the minimum effective dose to cause 90% mortality in the field) which was ascertained from previous field data and calculation of the MED_{90}/ED_{90} ratio (the ED_{90} was the minimum effective dose resulting in 90% mortality in the laboratory). From this ratio the multiplication factors necessary for determining field application rates were found. In this study no attempt has been made to determine field application rates, but the results indicate the likely field performance of hexythiazox.

Results from this study indicate that hexythiazox is highly toxic

to TSM eggs through both direct spray and residue exposure. In addition to ovicidal activity hexythiazox is reported to be highly toxic to the larval and nymphal stages of TSM (Anon., 1984; Chapman, 1986) and high mortality of both eggs, larval and nymphal stages would be expected in the field at suggested field application rates.

Hexythiazox was found to be of low toxicity to adult female TSM. Negligible mortality would be expected following spray applications in the field and feeding damage would continue. However, overall control of TSM is likely to be satisfactory because of the high toxicity of hexythiazox to TSM eggs and immature stages. Moreover, hexythiazox has been shown to have significant chemosterilant activity.

Adult female TSM were found to produce fewer viable eggs after direct spray with hexythiazox. Although the chemosterilant effect of hexythiazox has been shown to diminish following the removal of mites from treated surfaces, this does not apply in the field. Although the residue from field spraying would diminish as chemical breakdown occurs, hexythiazox has been shown to have strong persistent action (Anon., 1984), so that adult female TSM continue to be exposed. Chapman and Marris (unpublished data) simulated the breakdown of hexythiazox by transferring adult female TSM onto leaf discs every two days on which progressively halved application rates were sprayed. Even after a 600-fold dilution from the field rate no eggs hatched, although egg mortality may have been a result of residual action of hexythiazox on the eggs as much as the chemosterilant effect. Therefore, although adult female TSM are not killed by hexythiazox they are effectively rendered non-reproductive by the chemosterilant activity as long as residue levels remain sufficiently high.

The toxicity of hexythiazox was found to be inversely related to temperature over a range of 15 to 30°C. Therefore, control of TSM would probably be enhanced by cooler temperatures. Although mean temperatures early in the growing season may be lower than 15°C, extrapolation of these results would suggest enhanced toxicity at cooler temperatures. This may be of particular value as early season control of TSM is generally seen as fundamental to successful control throughout the season.

An inverse relationship between egg age and toxicity was found.

Spray applications would therefore be most effective if timed to coincide with the early deposition of eggs, particularly early in the season when there is a degree of synchronisation in egg ages. In addition, hexythiazox is reported to be highly toxic to the larval and nymphal stages of TSM (Anon., 1984; Chapman, 1986). Spray applications timed to coincide with a predominance of these stages, in particular the highly susceptible larvae, may also be effective in controlling TSM numbers.

The high toxicity of hexythiazox to eggs, larvae and nymphs and the additional chemosterilant action means there is a wide biological target that can be attacked. This lessens the need for highly accurate timing of spray applications, especially in view of the high persistency of hexythiazox (Anon., 1984). The broad toxicity range of hexythiazox may be of benefit in late-season applications when there is little synchronisation between the different life stages.

Baseline toxicity data for hexythiazox against TSM eggs have been established. This is of particular value in early detection of resistance to hexythiazox in the event of control failures in the field, especially in species such as TSM which have a history of rapid development of resistance. Possible cross resistance to hexythiazox in TSM resistant to clofentezine on field-grown roses in Australia has recently been discovered (Chapman, pers. comm.).

The demonstrated significant effect of leaf type on the toxicity of hexythiazox to TSM eggs has two important practical ramifications. Firstly, it highlights the need for standardisation of bioassay techniques so that variables other than those intended to be tested are minimised. Thus for any valid comparison to be made between leaf disc bioassay results, use of the same leaf type is necessary. Secondly, these results indicate the need for evaluation of a product, such as hexythiazox, over a range of TSM host plants to ensure that the desired control can be achieved.

CHAPTER 6
CONCLUSIONS

The following conclusions have been made from this study:

1. Hexythiazox is effectively non-toxic to adult female TSM. The LC_{50} value calculated from slide dip experiments was 59 times greater than the suggested field rate.

2. Hexythiazox was found to have a significant sterilising effect on adult female TSM resulting in reduced survival of deposited eggs. However, the sterilising effect was temporary. Egg survival recovered to control levels following removal of mites from hexythiazox residues.

In addition, direct spraying of adult female TSM resulted in significantly reduced oviposition rates compared with controls.

3. Baseline toxicity data were established for TSM eggs due to direct spray and residue exposure to hexythiazox. Calculated LC_{50} values were:

Direct spray exposure 1.6×10^{-4}% a.i.

Residue exposure 3.2×10^{-4}% a.i.

4. Over a temperature range of 15 to 30°C an inverse temperature-toxicity relationship was found to occur. An 8.7 fold difference in toxicity occurred over the temperature range studied.

5. The susceptibility of TSM eggs treated with hexythiazox was found to decrease with age. A four-fold decrease in susceptibility of eggs treated over ages ranging from 0 to 72 hours occurred. However, eggs treated over the 72 to 96 hour age range showed a marked increase in tolerance. This was thought to be due to closeness of treatment age and hatch point as there may not have been sufficient time available for hexythiazox to penetrate the egg and kill the embryo.

6. Leaf type was found to significantly affect the toxicity of hexythiazox to TSM eggs. A maximum 6.2 fold difference occurred between broad bean and 'Granny Smith' apple.

There was no apparent correlation between leaf surface structure and toxicity. It was suggested that toxicity differences may be attributable to variation in the ability of the leaf in transporting spray residues across its surface.

The use of a standardised leaf type for use in bioassy experiments was considered important so that variation due to this factor is minimised, thus allowing more valid comparison of results.

Assessment of required field rate application levels may also consider the effect of leaf type (i.e., the particular crop) on toxicity.

7. Light and scanning electron microscope examination of TSM eggs treated with a lethal dose of hexythiazox showed that embryos reach an advanced stage of development before death occurs. Killed embryos were indistinguishable from newly emerged larvae.

As other studies have shown hexythiazox to act on the chyrsalis stage of immature mites it was considered that the mode of action may involve inhibition of physiological processes common to the late stages of development of the egg and/or embryo and moulting in immature mites.

ACKNOWLEDGEMENTS

I wish to thank Mr R.B. Chapman for his excellent supervision of this study. His constructive criticism and helpful suggestions have been invaluable. I would also like to thank my associate supervisor Professor D.R. Penman for his assistance in administrative matters.

Thanks are due to Mr M.H. Bowie for technical assistance, Ms M.L. Barrell for comments on the draft manuscript, Ms S.M. Trotter and Mrs J.G. Hayes for their competent typing of this thesis. In addition I am most grateful to all the post-graduate students and staff of the Entomology Department for their friendship, interest and encouragement.

Financial support from May and Baker New Zealand Ltd., the Leonard Condell Foundation and the Charles and Ella Elgar Trust is gratefully acknowledged.

Hexythiazox was kindly supplied by May and Baker New Zealand Ltd.

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APPENDIX I

Common Acaricide Groups: Their Ovicidal Activity and Modes of Action

Chemical Group Common names of examples with ovicidal activity	Ovicidal Activity (LC_{50}, % a.i.)	Active against motile stages		Mode of Action	Comments	References
		Immatures	Adults			
Petroleum Oils	Not available	Yes	Yes	Physical action on the chorion leading to inhibition of respiration.	Primarily ovicidal in action. Commonly used as late winter or green tip spray against overwintering eggs of *Panonychus ulmi* and *Bryobia* spp. Due to phytotoxicity lighter more refined oils are used for summer applications. No resistance to oils has developed, probably due to physical mode of action.	Cranham and Helle (1985) Green et al. (1977) Jeppson et al. (1975) Smith and Pearce (1948) Smith and Salkeld (1966)
Organophosphorous Compounds ethion malathion parathion	Not available	Yes	Yes	Inhibition of acetylcholin-esterase	Primarily insecticidal com-pounds, some with secondary acaricidal activity. Although mainly active against motile forms, some have ovicidal action. Widespread resistance to these chemicals in tetranychid mites.	Corbett et al. (1984) Cremlyn and Cronje (1978) Hartley and Kidd (1983) Hintz (1953) Smith and Salkeld (1966)
Carbamates	_____	Yes	Yes	Inhibition of acetylcholin-esterase	Primarily insecticidal com-pounds. Some examples are active against mites (e.g., aldicarb, carbofuran, methomyl), but not active against eggs.	Corbett et al. (1984) Cranham anmd Helle (1985) Hartley and Kidd (1983)

Synthetic Pyrethroids	Not available	Yes	Yes	Poorly understood. A form of axonal inhibition.	Mainly insecticidal. Few examples active against motile mite forms. Some evidence of ovicidal activity.	Beeman (1982) Chapman (pers. comm.) Cranham and Helle (1985)
Organotin Compounds	_____	Yes	Yes	Inhibition of mitochondrial ATPase	Specific acaricides commonly used. Active against mite forms but not against eggs.	Corbett et al. (1984) Hartley and Kidd (1983)
Organochlorine Compounds dienochlor	Not available	Yes	Yes	Dienochlor : causes excessive release of acetylcholinesterase leading to hyperactivity of cholinergic synapses.	Mainly insecticidal compounds with low acaricidal activity. Dienochlor is an exception - a specific acaricide, active against all stages but predominantly ovicidal. There is also evidence of some chemosterilant action.	Corbett et al. (1984) Cremlyn and Cronje (1978) Hartley and Kidd (1983) Worthing (1983)
Bridged Diphenyl Compounds chlorobenzilate dicofol	 $7.8–12.6 \times 10^{-2}$ $6.0–12.0 \times 10^{-4}$	Yes	Yes	Unknown.	Specific acaricides, practically no insecticidal properties. Generally active against all mite stages including eggs.	Abul-Hab and Stafford (1956) Barker and Maughan (1956) Corbett et al. (1984) Cremlyn and Cronje (1978) Ebeling and Pence (1954) Jeppson et al. (1955) March (1958)

Sulphur Derivatives		Yes	No	Inhibition of mitochondrial ATPase.	Specific acaricides, chemically related to bridged diphenyl compounds. Typically active against eggs and larval stages but not adults.	Corbett et al. (1984) Cranham and Helle (1985) Ebeling and Pence (1954) Harrison and Smith (1961) Hartley and Kidd (1983) Henneberry et al. (1960) Jeppson et al. (1975) Meltzer and Dietvorst (1957) Overmeer (1967)
chlorbenside	$1.1\text{-}2.1 \times 10^{-3}$					
chlorfenson	5.7×10^{-4}					
tetradifon	$1.1\text{-}2.9 \times 10^{-4}$					
tetrasul	1.0×10^{-5}					
propargite	_____	Yes	Yes	Unknown	Propargite is a sulphur compound unrelated in structure to the bridged diphenyl compounds. It is active against motile stages only.	
Dinitrophenol Compounds	Not available	Yes	Yes	Uncoupling of oxidative phosphorylation causing inhibition of glycolysis.	Wide range of uses including acaricidal, insecticidal, herbicidal and fungicidal action. Activity against different mite stages variable. Binapacryl and dinocap are active against all stages including eggs. DCNP and DNOC are primarily ovicidal. Dinobuton is mainly active against motile stages. Herbicidal action of earlier forms limited their use to dormant sprays.	Buchell (1977) Corbett et al. (1984) Hartley and Kidd (1983) Lathrop and Hilborn (1957) Smith and Salkeld (1966) Ware (1978) Worthing (1983)
binapacryl						
DCNP						
dinobuton						
dinocap						
DNOC						

Formamidine Compounds amitraz chlordimeform formetanate	Not available	Yes	Yes	Action due to binding to the receptors of octopamine, a neurotransmission chemical.	Characterised by acaricidal activity. Activity against different mite stages varies. Chlordimeform is primarily ovicidal but also active against motile forms. Amitraz is active against all forms while formetanate is active against motile forms. Chlordimeform has been found to kill eggs by vapour action.	Buchell (1977) Corbett et al. (1984) Cranham and Helle (1985) Cremyln and Cronje (1978) Dittrich (1966, 1969) Gemrich et al. (1976) Hartley and Kidd (1983) Ware (1978)
Heterocyclic Compounds fenazoflor quinomethionate thioquinox	Not available	Yes	Yes	Unknown	Active as acaricides and fungicides. Active against eggs and motile forms. Quinomethionate is mainly ovicidal and larvacidal while thioquinox is mainly larvacidal.	Buchell (1977) Corbett et al. (1984) Cranham and Helle (1985) Hartley and Kidd (1983)
Fungicides benomyl karathane mancozeb propineb	Not available	No	No	Unknown	Several fungicides have secondary acaricidal activity. Benomyl and karathane both have ovicidal action while mancozeb and propineb have been found to reduce fecundity.	Corbett et al. (1977) Cranham and Helle (1985)

Cyclopropane Derivatives cylcoprate	4.0×10^{-3}	Yes	No	Interference of fat oxidation due to sequestration of carnitine a chemical that acts as a carrier of fatty acids.	Derived from juvenile hormone analogues cycloprate is an example of a class of compounds containing a cyclopropane moiety. A specific acaricide cycloprate is primarily active against eggs and larvae but essentially non-toxic to deutonymphs and adults. Chemosterilant action has been reported.	Asano and Kamei (1977) Corbett et al. (1984) Nelson and Show (1975) Staal et al. (1975)
Tetrazine Compounds clofentezine	1.6×10^{-5}	Yes	No	Unknown	Clofentezine is the only commercially produced acaricide in the tetrazine group. A specific acaricide, it is active against eggs, larvae and protonymphs but is non-toxic to deutonymphs and adults. Chlofentezine also has chemosterilant activity.	Anon. (1983) Aveyard et al. (1986) Chapman and Marris (1986) Read (1983)
Thiazolidinone Compounds hexythiazox	3.4×10^{-5}	Yes	No	Unknown	Hexythiazox is a specific acaricide of unique chemical structure. It is active against eggs and all immature forms but is non-toxic to adults. Hexythiazox also has chemosterilant activity.	Anon. (1984) Chapman (1986) Chapman and Marris (1986) Hoy and Quyang (1986)

APPENDIX II

Concentration-Mortality Data

Table 1 : Mortality of adult female TSM treated with hexythiazox using the slide dip method.

Concentration*	Number Tested	Mortality
5.0	40	40
4.0	40	40
2.0	40	36
1.8	40	36
1.5	80	45
1.0	40	8
0.8	40	5
0.5	40	8
0.48	40	4
0.0	120	10

* Concentration expressed in grams a.i. L^{-1}

Table 2 : Mortality of TSM eggs treated by direct spraying and residue exposure with hexythiazox using the leaf disc method.

Treatment	Concentration*	Number Tested	Mortality
Direct Spray	5.0	341	333
	4.0	472	346
	3.5	300	186
	3.0	397	310
	2.5	372	308
	2.0	301	175
	1.5	313	239
	1.35	342	174
	1.2	301	138
	1.0	299	135
	0.85	305	34
	0.75	316	113
	0.6	444	36
	0.5	383	123
	0.4	295	129
	0.3	379	34
	0.0	1300	145

Residue Exposure	10.0	411	411
	7.0	246	207
	6.0	329	250
	5.0	406	398
	4.0	419	263
	3.5	229	79
	3.0	634	289
	2.5	408	300
	2.25	359	71
	2.0	245	82
	1.75	355	42
	1.5	208	27
	1.3	328	45
	1.2	218	31
	1.0	261	47
	0.7	180	9
	0.5	280	22
	0.0	1235	95

* Concentration expressed as % a.i. x 10^{-4}

Table 3 : Mortality of TSM eggs incubated at different post-treatment temperatures treated with hexythiazox using the leaf disc method.

Temperature (°C)	Concentration*	Number Tested	Mortality
15	1.0	443	388
	0.75	283	248
	0.5	158	115
	0.4	229	159
	0.2	272	143
	0.1	386	76
	0.0	517	106
20	5.0	170	165
	3.5	147	101
	3.0	345	324
	2.5	162	104
	2.0	288	240
	1.5	136	87
	1.0	502	304
	0.75	185	80
	0.6	276	109
	0.5	190	59
	0.35	166	55
	0.3	416	29
	0.25	143	11
	0.1	116	6
	0.0	1110	79

25	5.0	341	333
	4.0	472	346
	3.5	300	186
	3.0	397	310
	2.5	372	308
	2.0	301	175
	1.5	313	239
	1.35	342	174
	1.2	301	138
	1.0	299	135
	0.85	305	34
	0.75	316	113
	0.6	444	36
	0.5	383	123
	0.4	295	129
	0.3	379	34
	0.0	1300	145
30	10.0	254	235
	7.5	204	170
	6.0	185	107
	5.0	439	341
	4.0	247	122
	3.5	286	196
	3.0	342	165
	2.5	402	264
	2.0	369	178
	1.5	636	255
	1.25	331	116
	1.2	283	15
	1.0	186	117
	0.75	169	38
	0.6	703	62
	0.55	411	25
	0.5	114	31
	0.4	278	26
	0.0	1421	104

* Concentration expressed as % a.i. x 10^{-4}

Table 4 : Mortality of TSM eggs treated at different ages with hexythiazox using the leaf disc method.

Egg-Age (hrs)	Concentration*	Number Treated	Mortality
0-6	2.5	86	77
	1.75	177	102
	1.5	110	70
	1.25	136	66
	1.0	178	84
	0.85	193	34
	0.75	194	75
	0.5	186	67

	0.4	143	18
	0.35	170	16
	0.2	178	15
	0.0	368	20
6-12	4.0	122	87
	3.0	123	94
	2.0	313	246
	1.75	130	74
	1.5	232	139
	1.4	190	75
	1.25	166	46
	1.2	132	34
	1.1	180	89
	0.9	181	27
	0.8	142	26
	0.7	151	18
	0.65	190	56
	0.6	53	20
	0.45	116	9
	0.4	340	24
	0.3	88	11
	0.25	173	16
	0.2	96	24
	0.0	634	52
12-24	5.0	139	132
	3.5	200	91
	3.0	238	185
	2.5	134	122
	2.0	552	346
	1.5	110	55
	1.2	357	106
	1.1	194	79
	1.0	124	81
	0.9	123	42
	0.75	113	74
	0.7	251	63
	0.6	189	106
	0.5	149	62
	0.4	547	59
	0.3	90	15
	0.25	264	28
	0.2	121	34
	0.0	954	73
24-48	10.0	379	362
	7.0	335	330
	6.0	316	229
	5.0	503	344
	4.0	260	144
	3.5	564	370
	2.5	722	343
	2.0	292	82
	1.5	779	328
	1.0	491	151
	0.85	214	40
	0.75	149	106
	0.7	693	82
	0.5	255	57
	0.0	1280	87

48-72	20.0	330	277
	14.0	398	384
	10.0	910	663
	8.0	244	149
	7.0	331	187
	6.0	353	225
	5.0	197	87
	3.0	699	188
	2.0	824	169
	1.7	210	90
	1.5	270	121
	1.2	267	82
	1.0	445	66
	0.85	331	69
	0.75	216	40
	0.5	158	18
	0.0	1308	95
72-96	1500.0	342	200
	1000.0	234	713
	750.0	302	200
	600.0	207	110
	300.0	233	105
	150.0	222	72
	100.0	315	73
	75.0	217	72
	60.0	236	88
	30.0	147	29
	15.0	257	44
	14.0	282	25
	7.5	128	11
	0.3	225	14
	0.0	1038	54

* Concentration expressed as % a.i. x 10^{-4}

Table 5 : Mortality of TSM eggs on different leaf surfaces treated with hexythiazox using the leaf disc method.

Leaf Type	Concentration	Number Tested	Mortality
Broad Bean	6.0	199	155
	4.5	247	142
	3.5	128	80
	3.0	210	105
	2.5	107	51
	2.0	179	49
	1.5	143	28
	1.25	124	25
	1.0	196	12
	0.75	248	28
	0.0	583	36

Strawberry	25.0	336	288
	22.0	297	264
	20.0	384	313
	18.0	305	222
	17.0	255	144
	15.0	354	259
	12.5	308	176
	10.0	345	133
	8.0	357	108
	6.0	335	108
	3.5	363	41
	2.5	366	41
	1.5	197	25
	1.0	280	13
	0.75	279	27
	0.0	1041	37
Raspberry	20.0	235	200
	18.0	163	134
	15.0	264	213
	12.5	238	142
	10.0	398	181
	8.0	227	66
	7.5	419	189
	6.0	427	123
	5.0	295	63
	4.5	222	41
	2.5	192	21
	1.5	327	32
	0.75	306	34
	0.0	1086	68
Red Delicious	40.0	394	352
	35.0	398	324
	30.0	294	175
	25.0	285	207
	20.0	257	142
	15.0	462	147
	12.5	296	97
	10.0	453	131
	8.0	326	59
	7.5	303	115
	6.0	368	59
	5.0	320	35
	2.5	348	44
	1.5	434	19
	0.75	268	22
	0.0	889	46
Granny Smith	35.0	110	74
	30.0	196	169
	27.5	128	97
	25.0	343	158
	22.5	118	72
	20.0	299	169
	17.5	246	80
	15.0	206	76
	10.0	293	92
	7.5	437	74
	5.0	168	38

2.5	341	27
1.5	241	27
0.00	706	33

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* Concentration expressed as % a.i. x 10^{-4}